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Signed Andrew Gersey

Dated 10 May 2001

official use

07JUL00 E551040-1 D01298
P01/7700 0.00-0016684.3

Your reference
PCS10910WPM-PROV

06 JUL 2000

0016684.3

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Notes
Please type, or write in dark ink using CAPITAL letters. A prescribed fee is payable for a request for grant of a patent. For details, please contact the Patent Office (telephone 071-438 4700).

Rule 16 of the Patents Rules 1990 is the main rule governing the completion and filing of this form.

2 Do not give trading styles, for example, 'Trading as XYZ company', nationality or former names, for example, 'formerly (known as) ABC Ltd' as these are not required.

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The Patent Office

Request for grant of a Patent

Form 1/77

Patents Act 1977

1 Title of invention

1 Please give the title of the invention

PHARMACEUTICAL COMPOSITION

2 Applicant's details

☐ **First or only applicant**

2a If you are applying as a corporate body please give:

Corporate name
PFIZER LIMITED

Country *(and State of incorporation, if appropriate)*
UNITED KINGDOM

2b If you are applying as an individual or one of a partnership please give in full:

Surname

Forenames

2c **In all cases,** please give the following details:

Address
RAMSGATE ROAD
SANDWICH
KENT

UK postcode CT13 9NJ
(if applicable)

Country UNITED KINGDOM

ADP number *(if known)* 68926 73001

2d, and 2f:
If there are further applicants please provide details on a separate sheet of paper.

Second applicant (if any)

2d If you are applying as a corporate body please give:

Corporate name

Country *(and State of incorporation, if appropriate)*

2e If you are applying as an individual or one of a partnership please give in full:

Surname

Forenames

2f **In all cases,** please give the following details:

Address

UK postcode
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Country

ADP number
(if known)

3
An address for service in the United Kingdom must be supplied.
Please mark correct box

3 Address for service details

3a Have you appointed an agent to deal with your application?

Yes [X] No [ ] ➡ *go to 3b*

⬇ ***Please give details below***

Agent's name
DR. RICHARD C. SEWELL
Agent's address
PFIZER LIMITED
RAMSGATE ROAD
SANDWICH
KENT
Postcode CT13 9NJ

Agent's ADP number 6892673001

3b:
If you have appointed an agent, all correspondence concerning your application will be sent to the agent's United Kingdom address.

3b If you have not appointed an agent please give a name and address in the United Kingdom to which all correspondence will be sent:

Name

Address

Postcode
ADP number
(if known)

Daytime telephone number*(if available)*

4 Refer nce number

4 Agent's or applicant's reference number *(if applicable)* PCS10910WPM-PROV

5 Claiming an earlier application date

5 Are you claiming that this application be treated as having been filed on the date of filing of an earlier application?

Please mark correct box

Yes ☐ No [X] ➡ *go to 6*

⬇ *please give details below*

❑ number of earlier application or patent number

❑ filing date

(day month year)

❑ and the Section of the Patents Act 1977 under which you are claiming:

Please mark correct box

15(4) (Divisional) ☐ 8(3) ☐ 12(6) ☐ 37(4) ☐

6 Declaration of priority

f you are declaring priority from a 'CT Application please enter 'PCT' is the country and enter the country ode (for example, GB) as part of the ıpplication number.

'lease give the date in all number format, for example, 31/05/90 for 31 May 1990.

6 If you are declaring priority from previous application(s), please give:

Country of filing	Priority application number *(if known)*	Filing date *(day,month,year)*

he answer must be 'No' if:
any applicant is not an inventor
there is an inventor who is not an applicant, or
any applicant is a corporate body.

lease supply duplicates of laim(s), abstract, description nd drawing(s).

lease mark correct box(es)

)

ou or your appointed agent see Rule 90 of the Patents Rules 1990) must sign this equest.

Please sign here

A completed fee sheet should preferably accompany the fee.

7 Inventorship

7 Are you (the applicant or applicants) the sole inventor or the joint inventors?

Please mark the correct box

Yes [ ] No [X]

A statement of Inventorship on Patents Form 7/77 will need to be filed (see Rule 15).

8 Checklist

8a Please fill in the number of sheets for each of the following types of document contained in this application.

Continuation sheets for this Patents Form 1/77			
Claim(s)	11	Description	102
Abstract		Drawing(s)	

8b Which of the following documents also accompanies the application?

Priority documents *(please state how many)*	
Translation(s) of Priority documents *(please state how many)*	
Patents Form 7/77 - Statement of Inventorship and Right to Grant *(please state how many)*	
Patents Form 9/77 - Preliminary Examination/Search	
Patents Form 10/77 - Request for Substantive Examination	

9 Request

I/We request the grant of a patent on the basis of this application.

Signed Richard Sewell Date 06/07/2000
(day month year)

Please return the completed form, attachments and duplicates where requested, together with the prescribed fee to:

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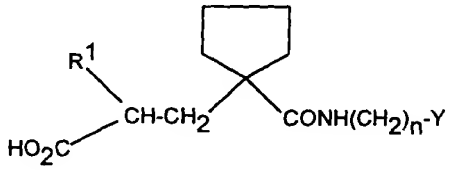
Pharmaceutical Composition

This invention relates to inhibitors of neutral endopeptidase enzyme (NEP) and derivatives thereof and to processes for the preparation of, intermediates used in the preparation of, compositions containing and the uses of said inhibitors. These inhibitors have utilility in a variety of therapeutic areas including the treatment of sexual disorders in particular female sexual dysfunction, especially wherein the female sexual dysfunction treated includes female sexual arousal disorder.

NEP inhibitors are disclosed in WO 91/07386 and WO 91/10644.

According to a first aspect, the present invention provides the use of a compound of formula (I), or a pharmaceutically acceptable salt, solvate or prodrug thereof, in the preparation of a medicament for the treatment of sexual dysfunction:



(I)

wherein

R^1 is C_{1-6}alkyl which may be substituted by one or more substituents, which may be the same or different, selected from the list: halo, hydroxy, C_{1-6} alkoxy, C_{2-6} hydroxyalkoxy, C_{1-6} alkoxy(C_{1-6}alkoxy), C_{3-7}cycloalkyl, C_{3-7}cycloalkenyl, aryl, aryloxy, (C_{1-4}alkoxy)aryloxy, heterocyclyl, heterocyclyloxy, $-NR^2R^3$, $-NR^4COR^5$, $-NR^4SO_2R^5$, $-CONR^2R^3$, $-S(O)_pR^6$, $-COR^7$ and $-CO_2(C_{1-4}alkyl)$; or R^1 is C_{3-7}cycloalkyl, aryl or heterocyclyl, each of which may be substituted by one or more substituents from said list, which substituents may be the same or different, which list further includes C_{1-6}alkyl; or R^1 is C_{1-6} alkoxy, $-NR^2R^3$ or $-NR^4SO_2R^5$;

wherein

R^2 and R^3 are each independently H, C_{1-4}alkyl, C_{3-7}cycloalkyl (optionally substituted by hydroxy or C_{1-4}alkoxy), aryl, (C_{1-4}alkyl)aryl, C_{1-6}alkoxyaryl or heterocyclyl; or R^2 and R^3 together with the nitrogen to which they are attached form a pyrrolidinyl, piperidino, morpholino, piperazinyl or *N*-(C_{1-4} alkyl)piperazinyl group;

R^4 is H or C_{1-4}alkyl;

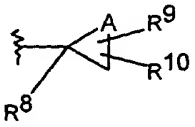
R^5 is C_{1-4}alkyl, CF_3, aryl, (C_{1-4} alkyl)aryl, (C_{1-4}alkoxy)aryl, heterocyclyl, C_{1-4}alkoxy or -NR^2R^3 wherein R^2 and R^3 are as previously defined;

R^6 is C_{1-4}alkyl, aryl, heterocyclyl or NR^2R^3 wherein R^2 and R^3 are as previously defined; and

R^7 is C_{1-4}alkyl, C_{3-7}cycloalkyl, aryl or heterocyclyl; n is 0, 1 or 2; p is 0, 1, 2 or 3;

the -$(CH_2)_n$- linkage is optionally substituted by C_{1-4}alkyl, C_{1-4}alkyl substituted with one or more fluoro groups or phenyl, C_{1-4}alkoxy, hydroxy, hydroxy(C_{1-3}alkyl), C_{3-7}cycloalkyl, aryl or heterocyclyl;

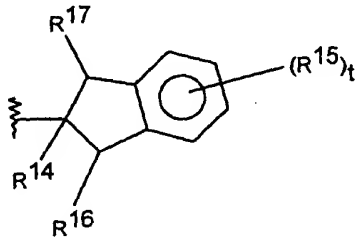
Y is the group



wherein A is -$(CH_2)_q$- where q is 1, 2, 3 or 4 to complete a 3 to 7 membered carbocyclic ring which may be saturated or unsaturated; R^8 is H, C_{1-6}alkyl, -CH_2OH, phenyl, phenyl(C_{1-4}alkyl) or $CONR^{11}R^{12}$; R^9 and R^{10} are each independently H, -CH_2OH, -$C(O)NR^{11}R^{12}$, C_{1-6}alkyl, phenyl optionally substituted by C_{1-4}alkyl, or phenyl(C_{1-4}alkyl) wherein the phenyl group is optionally substituted by C_{1-4}alkyl, or R^9 and R^{10} together form a dioxolane; $R^{11}$and R^{12} which may be the same or different are H, C_{1-4}alkyl, R^{13} or $S(O)_rR^{13}$, where r is 0, 1 or 2 and R^{13} is phenyl optionally substituted by C_{1-4}alkyl or phenylC_{1-4}alkyl wherein the phenyl is optionally substituted by C_{1-4}alkyl; or

Y is the group, -C(O) NR^{11} R^{12} wherein R^{11} and R^{12} are as previously defined except that R^{11} and R^{12} are not both H; or

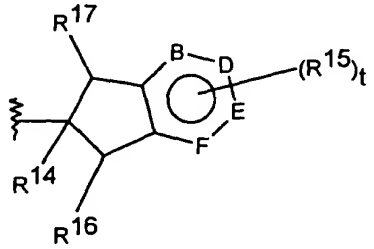
Y is the group,



wherein R^{14} is H, CH_2OH, or $C(O)NR^{11}R^{12}$ wherein R^{11} and R^{12} are as previously defined; when present R^{15}, which may be the same or different to

any other R^{15}, is OH, C_{1-4}alkyl, C_{1-4}alkoxy, halo or CF_3; t is 0, 1, 2, 3 or 4; and R^{16} and R^{17} are independently H or C_{1-4} alkyl; or

Y is the group



wherein one or two of B, D, E or F is a nitrogen, the others being carbon; and R^{14} to R^{17} and t are as previously defined; or

Y is an optionally substituted 5-7 membered heterocyclic ring, which may be saturated, unsaturated or aromatic and contains a nitrogen, oxygen or sulphur and optionally one, two or three further nitrogen atoms in the ring and which may be optionally benzofused and optionally substituted by:

C_{1-6} alkoxy; hydroxy; oxo; amino; mono or di-(C_{1-4}alkyl)amino; C_{1-4}alkanoylamino; or

C_{1-6}alkyl which may be substituted by one or more substituents, which may be the same or different, selected from the list: C_{1-6}alkoxy, C_{1-6}haloalkoxy, C_{1-6}alkylthio, halogen, C_{3-7}cycloalkyl, heterocyclyl or phenyl; or

C_{3-7}cycloalkyl, aryl or heterocyclyl, each of which may be substituted by one or more substituents, which may be the same or different, selected from the list: C_{1-6}alkyl, C_{1-6}alkoxy, C_{1-6}haloalkoxy, C_{1-6}alkylthio, halogen, C_{3-7}cycloalkyl, heterocyclyl or phenyl;

wherein when there is an oxo substitution on the heterocyclic ring, the ring only contains one or two nitrogen atoms and the oxo substitution is adjacent a nitrogen atom in the ring; or

Y is $-NR^{18}S(O)_uR^{19}$, wherein R^{18} is H or C_{1-4}alkyl; R^{19} is aryl, arylC_{1-4}alkyl or heterocyclyl (preferably pyridyl); and

u is 0, 1, 2 or 3.

Some of the compounds of formula I are disclosed in WO 91/10664 and WO 91/07386, but there is no teaching that they could be useful in the treatment of sexual dysfunction. The remaining compounds of formula I are nov l. Therefore according to a second aspect, the invention provides a (novel) compound of formula

(I), or a pharmaceutically acceptable salt, solvate or prodrug thereof, wherein R^1, n and Y are as defined in the first aspect with the proviso that Y is not the group $-C(O)NR^{11}R^{12}$ and R^{14} is not H or $-CH_2OH$.

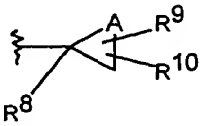
Unless otherwise specified, the compounds of the first and second aspects are hereinafter defined as compounds of the invention.

Where it is necessary to distinguish between the known and novel compounds of the invention, the novel compounds will be referred to as the compounds of the second aspect of the invention.

The following are preferred compounds of the invention, i.e. in accordance with the first and second aspects of the invention.

Preferably R^1 is C_{1-6}alkyl, C_{1-6}alkoxy, C_{1-6}alkoxy(C_{1-3})alkyl, C_{1-6}alkoxyC_{1-6}alkoxyC_{1-3}alkyl or C_{1-6}alkyl substituted with aryl. Particularly preferred R^1 substituents are C_{1-6}alkyl, C_{1-6}alkoxy, C_{1-6}alkoxy(C_{1-3})alkyl (especially methoxyethyl) or C_{1-6}alkoxyC_{1-6}alkoxyC_{1-3}alkyl (especially methoxyethoxymethyl). It is especially preferred that R^1 is C_{1-4}alkyl (preferably propyl).

When Y is the group



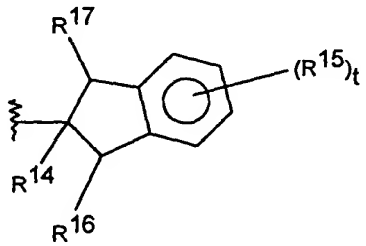
and the carbocyclic ring is fully saturated, then preferably one of R^9 or R^{10} is $-CH_2OH$, $-C(O)NR^{11}R^{12}$, C_{1-6}alkyl, phenyl optionally substituted by C_{1-4}alkyl or phenyl(C_{1-4}alkyl) wherein the phenyl group is optionally substituted by C_{1-4}alkyl. More, preferably the carbocyclic ring is 5, 6 or 7 membered wherein one of R^9 or R^{10}, $-C(O)NR^{11}R^{12}$, with the other being C_{1-6}alkyl, phenyl optionally substituted by C_{1-4}alkyl or phenyl(C_{1-4}alkyl) wherein the phenyl group is optionally substituted by C_{1-4}alkyl. More preferably, R^9 and R^{10} are attached to adjacent carbon atoms in the ring. More preferably, R^8 is CH_2OH.

When Y is the group $-NR^{18}S(O)_uR^{19}$, preferably R^{18} is H. More preferably, R^{19} is benzyl or phenyl. More preferably u is 2.

Preferably Y is the optionally substituted 5-7 membered heterocyclic ring. More preferably the ring is an optionally substituted aromatic ring, particularly pyridyl, pyrazinyl, pyrimidinyl, pyridazinyl, pyrazolyl, triazolyl, tetrazolyl, oxadiazolyl, thiazolyl, thiaiazolyl, oxazolyl, isoxazolyl, indolyl, isoindolinyl, quinolyl, isoquinolyl, pyridonyl, quinoxalinyl or quinazolinyl [especially oxadiazole (preferably 1,2,5- or 1,3,4-oxadiazole), pyridone (preferably 2-pyridone) or thiadiazole (preferably 1,3,4-thiadiazole) each of which may be substituted as defined in the first aspect. Preferably the heterocyclic ring is substituted by one or more C_{1-6}alkyl, phenyl or phenylC_{1-4}alkyl, more preferably by C_{1-4}alkyl or benzyl. Preferably Y is an *N*-substituted pyridone, preferably by benzyl or C_{1-4}alkyl.

Preferably Y is a lactam linked at the nitrogen.

Preferably Y is



wherein R^{14} is preferably CH_2OH or $C(O)NR^{11}R^{12}$, especially $C(O)NR^{11}R^{12}$. Preferably R^{16} and R^{17} are hydrogen. Preferably t is 0.

The chiral carbon attached to R^1 is preferably the R-enantiomer.

Particularly preferred compounds of the invention (referred to hereinafter as the list of 10 preferred compounds) are:
2-[(1-{[(1-benzyl-6-oxo-1,6-dihydro-3-pyridinyl)amino]carbonyl}cyclopentyl)methyl]-4-methoxybutanoic acid (Example 35),
2-{[1-({[3-(2-oxo-1-pyrrolidinyl)propyl]amino}carbonylcyclopentyl]-methyl}-4-phenylbutanoic acid (Example 40),
(+)-2-{[1-({[2-(hydroxymethyl)-2,3-dihydro-1*H*-inden-2-yl]amino}carbonyl)cyclopentyl]-methyl}-4-phenylbutanoic acid (Example 44),

2-[(1-{[(5-methyl-1,3,4-thiadiazol-2-yl)amino]carbonyl}cyclopentyl)methyl]-4-phenylbutanoic acid (Example 43),
cis-3-(2-methoxyethoxy)-2-[(1-{[(4-{[(phenylsulfonyl)amino]carbonyl}cyclohexyl)-amino]carbonyl}cyclopentyl)methyl]propanoic acid (Example 38),
(+)-2-{[1-({[2-(hydroxymethyl)-2,3-dihydro-1*H*-inden-2-yl]amino}carbonyl)cyclopentyl]-methyl}pentanoic acid (Example 31),
(+)-2-[(1-{[(5-ethyl-1,3,4-thiadiazol-2-yl)amino]carbonyl}cyclopentyl)methyl]pentanoic acid (Example 30),
2-({1-[(3-benzylanilino)carbonyl]cyclopentyl}methyl)pentanoic acid (Example 21),
2-[(1-{[(1-benzyl-6-oxo-1,6-dihydro-3-pyridinyl)amino]carbonyl}cyclopentyl)methyl]-pentanoic acid (Example 22), and
2-{[1-({[(1R,3S,4R)-4-(aminocarbonyl)-3-butylcyclohexyl]amino}carbonyl)-cyclopentyl]methyl}pentanoic acid (Example 9).

In the above definition, unless otherwise indicated, alkyl groups having three or more carbon atoms may be straight or branched-chain. The term aryl as used herein means an aromatic hydrocarbon group such as phenyl or naphthyl which may optionally be substituted with, for example, one or more of OH, CN, CF_3, C_1-C_4 alkyl, C_1-C_4 alkoxy, halo, carbamoyl, aminosulphonyl, amino, mono or di(C_1-C_4 alkyl)amino or (C_1-C_4 alkanoyl)amino groups. Halo means fluoro, chloro, bromo or iodo.

In the above definition, unless otherwise indicated the term heterocyclyl means a 5 or 6 membered nitrogen, oxygen or sulphur containing heterocyclic group which, unless otherwise stated, may be saturated, unsaturated or aromatic and which may optionally include a further oxygen or one to three nitrogen atoms in the ring and which may optionally be benzofused or substituted with for example, one or more halo, C_1-C_4 alkyl, hydroxy, carbamoyl, benzyl, oxo, amino or mono or di-(C_1-C_4 alkyl)amino or (C_1-C_4 alkanoyl)amino groups. Particular examples of heterocycles include pyridyl, pyridonyl, pyrazinyl, pyrimidinyl, pyridazinyl, pyrrolyl, imidazolyl, pyrazolyl, triazolyl, tetrazolyl, furanyl, tetrahydrofuranyl, tetrahydropyranyl, dioxanyl, thienyl, oxazolyl, isoxazolyl, thiazolyl, oxadiazolyl, thiadiazolyl, indolyl, isoindolinyl, quinolyl, isoquinolyl, quinoxalinyl, quinazolinyl and benzimidazolyl, each being optionally substituted as previously defined.

The compounds of the invention are inhibitors of the zinc-dependent, neutral endopeptidase EC.3.4.24.11., and it is proposed that the compounds of the invention will treat the disease states listed below. This enzyme is involved in the breakdown of several bioactive oligopeptides, cleaving peptide bonds on the amino side of hydrophobic amino acid residues. The peptides metabolised include atrial natriuretic peptides (ANP), bombesin, bradykinin, calcitonin gene-related peptide, endothelins, enkephalins, neurotensin, substance P and vasoactive intestinal peptide. Some of these peptides have potent vasodilatory and neurohormone functions, diuretic and natriuretic activity or mediate behaviour effects. Thus, the compounds of the invention, by inhibiting the neutral endopeptidase EC.3.4.24.11, can potentiate the biological effects of bioactive peptides. Thus, in particular the compounds have utility in the treatment of a number of disorders, including hypertension, heart failure, angina, renal insufficiency, cyclical oedema, Menières disease, hyperaldosteroneism (primary and secondary) and hypercalciuria. In addition, because of their ability to potentiate the effects of ANF the compounds have utility in the treatment of glaucoma. As a further result of their ability to inhibit the neutral endopeptidase E.C.3.4.24.11 the compounds of the invention may have activity in other therapeutic areas including for example the treatment of menstrual disorders, preterm labour, pre-eclampsia, endometriosis, and reproductive disorders (especially male and female infertility, polycystic ovarian syndrome, implantation failure). Also the compounds of the invention should treat asthma, inflammation, leukemia, pain, epilepsy, affective disorders, dementia and geriatric confusion, obesity and gastrointestinal disorders (especially diarrhoea and irritable bowel syndrome), wound healing (especially diabetic and venous ulcers and pressure sores), septic shock, the modulation of gastric acid secretion and the treatment of hyperreninaemia. In a preferred embodiment the compounds of the invention are useful in the treatment of male and female sexual dysfunction.

We have found that the compounds of the invention inhibit the enzyme neutral endopeptidase. Therefore, according to a further aspect, the invention provides the use of a compound as defined in the second aspect in the preparation of a medicament for the treatment or prophylaxis of a condition for which a beneficial therapeutic response can be obtained by the inhibition of neutral ndopeptidase.

The compounds of the invention are particularly beneficial for the treatment of sexual dysfunction in the male (e.g. male erectile dysfunction), more particularly in the female - female sexual dysfunction (FSD).

In accordance with the invention, FSD can be defined as the difficulty or inability of a woman to find satisfaction in sexual expression. FSD is a collective term for several diverse female sexual disorders (Leiblum, S.R. (1998). Definition and classification of female sexual disorders. *Int. J. Impotence Res.*, **10**, S104-S106; , Berman, J.R., Berman, L. & Goldstein, I. (1999). Female sexual dysfunction: Incidence, pathophysiology, evaluations and treatment options. *Urology*, **54**, 385-391). The woman may have lack of desire, difficulty with arousal or orgasm, pain with intercourse or a combination of these problems. Several types of disease, medications, injuries or psychological problems can cause FSD. Treatments in development are targeted to treat specific subtypes of FSD, predominantly desire and arousal disorders.

The categories of FSD are best defined by contrasting them to the phases of normal female sexual response: desire, arousal and orgasm (Leiblum, S.R. (1998). Definition and classification of female sexual disorders. *Int. J. Impotence Res.*, 10, S104-S106). Desire or libido is the drive for sexual expression. Its manifestations often include sexual thoughts either when in the company of an interested partner or when exposed to other erotic stimuli. Arousal is the vascular response to sexual stimulation, an important component of which is genital engorgement and includes increased vaginal lubrication, elongation of the vagina and increased genital sensation/sensitivity. Orgasm is the release of sexual tension that has culminated during arousal.

Hence, FSD occurs when a woman has an inadequate or unsatisfactory response in any of these phases, usually desire, arousal or orgasm. FSD categories include hypoactive sexual desire disorder, sexual arousal disorder, orgasmic disorders and sexual pain disorders. Although the compounds of the invention will improve the genital response to sexual stimulation (as in female sexual arousal disorder), in doing so it may also improve the associated pain, distress and discomfort associated with intercourse and so treat other female sexual disorders.

Thus, in accordance with a preferred aspect of the invention, there is provided use of a compound of the invention in the pr paration of a medicament for the treatment or

prophylaxis of hypoactive sexual desire disorder , sexual arousal disorder, orgasmic disorder and sexual pain disorder, more preferably for the treatment or propylaxis of sexual arousal disorder, orgasmic disorder, and sexual pain disorder, and most preferably in the treatment or prophylaxis of sexual arousal disorder.

Hypoactive sexual desire disorder is present if a woman has no or little desire to be sexual, and has no or few sexual thoughts or fantasies. This type of FSD can be caused by low testosterone levels, due either to natural menopause or to surgical menopause. Other causes include illness, medications, fatigue, depression and anxiety.

Female sexual arousal disorder (FSAD) is characterised by inadequate genital response to sexual stimulation. The genitalia do not undergo the engorgement that characterises normal sexual arousal. The vaginal walls are poorly lubricated, so that intercourse is painful. Orgasms may be impeded. Arousal disorder can be caused by reduced oestrogen at menopause or after childbirth and during lactation, as well as by illnesses, with vascular components such as diabetes and atherosclerosis. Other causes result from treatment with diuretics, antihistamines, antidepressants eg SSRIs or antihypertensive agents.

Sexual pain disorders (includes dyspareunia and vaginismus) is characterised by pain resulting from penetration and may be caused by medications which reduce lubrication, endometriosis, pelvic inflammatory disease, inflammatory bowel disease or urinary tract problems.

The prevalence of FSD is difficult to gauge because the term covers several types of problem, some of which are difficult to measure, and because the interest in treating FSD is relatively recent. Many women's sexual problems are associated either directly with the female ageing process or with chronic illnesses such as diabetes and hypertension.

Because FSD consists of several subtypes that express symptoms in separate phases of the sexual response cycle, there is not a single therapy. Current treatment of FSD focuses principally on psychological or relationship issues. Treatment of FSD is gradually evolving as more clinical and basic science studies are dedicated to the

investigation of this medical problem. Female sexual complaints are not all psychological in pathophysiology, especially for those individuals who may have a component of vasculogenic dysfunction (eg FSAD) contributing to the overall female sexual complaint. There are at present no drugs licensed for the treatment of FSD. Empirical drug therapy includes oestrogen administration (topically or as hormone replacement therapy), androgens or mood-altering drugs such as buspirone or trazodone. These treatment options are often unsatisfactory due to low efficacy or unacceptable side effects.

Since interest is relatively recent in treating FSD pharmacologically, therapy consists of the following:- psychological counselling, over-the-counter sexual lubricants, and investigational candidates, including drugs approved for other conditions. These medications consist of hormonal agents, either testosterone or combinations of oestrogen and testosterone and more recently vascular drugs, that have proved effective in male erectile dysfunction. None of these agents has been demonstrated to be very effective in treating FSD.

As discussed, the compounds of the invention are particularly useful for the treatment of female sexual arousal disorder (FSAD).

The Diagnostic and Statistical Manual (DSM) IV of the American Psychiatric Association defines Female Sexual Arousal Disorder (FSAD) as being:

> *"a persistent or recurrent inability to attain or to maintain until completion of the sexual activity adequate lubrication-swelling response of sexual excitement. The disturbance must cause marked distress or interpersonal difficulty."*

The arousal response consists of vasocongestion in the pelvis, vaginal lubrication and expansion and swelling of the external genitalia. The disturbance causes marked distress and/or interpersonal difficulty.

FSAD is a highly prevalent sexual disorder affecting pre-, peri- and post menopausal (±HRT) women. It is associated with concomitant disorders such as depression, cardiovascular dis ases, diabetes and UG disorders.

The primary consequences of FSAD are lack of engorgement/swelling, lack of lubrication and lack of pleasurable genital sensation. The secondary consequences of FSAD are reduced sexual desire, pain during intercourse and difficulty in achieving an orgasm.

It has recently been hypothesised that there is a vascular basis for at least a proportion of patients with symptoms of FSAD (Goldstein *et al.*, Int. J. Impot. Res., 10, S84-S90,1998) with animal data supporting this view (Park *et al.*, Int. J. Impot. Res., 9, 27-37, 1997).

Drug candidates for treating FSAD, which are under investigation for efficacy, are primarily erectile dysfunction therapies that promote circulation to the male genitalia. They consist of two types of formulation, oral or sublingual medications (Apomorphine, Phentolamine, phosphodiesterase type 5 (PDE5) inhibitors e.g. Sildenafil), and prostaglandin (PGE_1) that are injected or administered transurethrally in men, and topically to the genitalia in women.

The present invention is advantageous as it provides a means for restoring a normal sexual arousal response - namely increased genital blood flow leading to- vaginal, clitoral and labial engorgement. This will result in increased vaginal lubrication *via* plasma transudation, increased vaginal compliance and increased genital sensitivity. Hence, the present invention provides a means to restore, or potentiate, the normal sexual arousal response.

Without being bound by theory, we believe that neuropeptides such as vasoactive intestinal peptide (VIP) are major neurotransmitter candidates in the control of the female sexual arousal response, especially in the control of genital blood flow. VIP and other neuropeptides are degraded/ metabolised by NEP EC3.4.24.11. Thus, NEP inhibitors will potentiate the endogenous vasorelaxant effect of VIP released during arousal. This will lead to a treatment of FSAD, such as through enhanced genital blood flow and hence genital engorgement. We have shown that selective inhibitors of NEP EC 3.4.24.11 enhance pelvic nerve-stimulated and VIP-induced increases in vaginal and clitoral blood flow. In addition, selective NEP inhibitors enhance VIP and nerve-mediated relaxations of isolated vagina wall.

Thus the present invention is advantageous as it helps provide a means for restoring a normal sexual arousal response - namely increased genital blood flow leading to vaginal, clitoral and labial engorgement. This will result in increased vaginal lubrication *via* plasma transudation, increased vaginal compliance and increased vaginal sensitivity. Hence, the present invention provides a means to restore, or potentiate the normal sexual arousal response.

Background teachings on NEP have been presented by Victor A. McKusick et al on http://www3.ncbi.nlm.nih.gov/Omim/searchomim.htm. The following information concerning NEP has been extracted from that source.

> "Common acute lymphocytic leukemia antigen is an important cell surface marker in the diagnosis of human acute lymphocytic leukemia (ALL). It is present on leukemic cells of pre-B phenotype, which represent 85% of cases of ALL. CALLA is not restricted to leukemic cells, however, and is found on a variety of normal tissues. CALLA is a glycoprotein that is particularly abundant in kidney, where it is present on the brush border of proximal tubules and on glomerular epithelium. Letarte et al. (1988) cloned a cDNA coding for CALLA and showed that the amino acid sequence deduced from the cDNA sequence is identical to that of human membrane-associated neutral endopeptidase (NEP; EC 3.4.24.11), also known as enkephalinase. NEP cleaves peptides at the amino side of hydrophobic residues and inactivates several peptide hormones including glucagon, enkephalins, substance P, neurotensin, oxytocin, and bradykinin. By cDNA transfection analysis, Shipp et al. (1989) confirmed that CALLA is a functional neutral endopeptidase of the type that has previously been called enkephalinase. Barker et al. (1989) demonstrated that the CALLA gene, which encodes a 100-kD type II transmembrane glycoprotein, exists in a single copy of greater than 45 kb which is not rearranged in malignancies expressing cell surface CALLA. The gene was located to human chromosome 3 by study of somatic cell hybrids and in situ hybridization regionalized the location to 3q21-q27. Tran-Paterson et al. (1989) also assigned the gene to chromosome 3 by Southern blot analysis of DNA from human-rodent somatic cell hybrids. D'Adamio et al. (1989) demonstrated that the CALLA gene spans more than 80 kb and is composed of 24 exons."

1. Barker, P. E.; Shipp, M. A.; D'Adamio, L.; Masteller, E. L.; Reinherz, E. L. The common acute lymphoblastic leukemia antigen gene maps to chromosomal region 3(q21-q27). J. Immun. 142: 283-287, 1989.

2. D'Adamio, L.; Shipp, M. A.; Masteller, E. L.; Reinherz, E. L. : Organization of the gene encoding common acute lymphoblastic leukemia antigen (neutral endopeptidase 24.11): multiple miniexons and separate 5-prime untranslated regions. Proc. Nat. Acad. Sci. 86: 7103-7107, 1989.

3. Letarte, M.; Vera, S.; Tran, R.; Addis, J. B. L.; Onizuka, R. J.; Quackenbush, E. J.; Jongeneel, C. V.; McInnes, R. R. : Common acute lymphocytic leukemia antigen is identical to neutral endopeptidase. J. Exp. Med. 168: 1247-1253, 1988.

4. Shipp, M. A.; Vijayaraghavan, J.; Schmidt, E. V.; Masteller, E. L.; D'Adamio, L.; Hersh, L. B.; Reinherz, E. L. : Common acute lymphoblastic leukemia antigen (CALLA) is active neutral endopeptidase 24.11 ('enkephalinase'): direct evidence by cDNA transfection analysis. Proc. Nat. Acad. Sci. 86: 297-301, 1989.

5. Tran-Paterson, R.; Willard, H. F.; Letarte, M. : The common acute lymphoblastic leukemia antigen (neutral endopeptidase--3.4.24.11) gene is located on human chromosome 3. Cancer Genet. Cytogenet. 42: 129-134, 1989.

The compounds of formula (I) may contain several asymmetric centres and thus they can exist an enantiomers and diastereomers. The invention includes both the separated individual isomers as well as mixutres of isomers.

The pharmaceutically or veterinarily acceptable salts of the compounds of the invention which contain a basic centre are, for example, non-toxic acid addition salts formed with inorganic acids such as hydrochloric, hydrobromic, hydroiodic, sulphuric and phosphoric acid, with carboxylic acids or with organo-sulphonic acids. Examples include the HCl, HBr, HI, sulphate or bisulphate, nitrate, phosphate or hydrogen phosphate, acetate, benzoate, succinate, saccarate, fumarate, maleate, lactate, citrate, tartrate, gluconate, camsylate, methanesulphonate, ethanesulphonate, benzenesulphonate, p-toluenesulphonate and pamoate salts. The pharmaceutically or veterinarily acceptable salts of the compounds of the invention which contain an acidic

centre are those formed with bases which form non-toxic salts. Examples include the alkali metal salts such as the sodium, potassium or calcium salts or salts with amines such as diethylamine and dicyclohexylamine. For a review on suitable pharmaceutical salts see Berge et al, J. Pharm, Sci., 66, 1-19, 1977.

The pharmaceutically acceptable solvates of the compounds of the invention include the hydrates thereof. All polymorphs of the compounds of the invention are also included within the scope of the invention.

In cases where the compounds of the invention exist as the E and Z isomers, the invention includes individual isomers as well as mixtures thereof.

In cases where compounds of the invention exist as tautomeric isomers, the invention includes individual tautomers as well as mixtures thereof.

In cases where the compounds of the invention exist as optical isomers, the invention includes individual isomers as well as mixtures thereof.

In cases where the compounds of the invention exist as diastereoisomers, the invention includes individual diastereoisomers as well as mixtures thereof.

Separation of diastereoisomers or E and Z isomers may be achieved by conventional techniques, e.g. by fractional crystallisation, chromatography or H.P.L.C. An individual enantiomer of a compound of the invention may also be prepared from a corresponding optically pure intermediate or by resolution, such as by H.P.L.C. of the corresponding racemate using a suitable chiral support or by fractional crystallisation of the diastereoisomeric salts formed by reaction of the corresponding racemate with a suitable optically active acid or base, as appropriate.

The present invention also includes all suitable isotopic variations of compounds of the invention or pharmaceutically acceptable salt thereof. An isotopic variation of compounds of the invention or a pharmaceutically acceptable salt thereof is defined as one in which at least one atom is replaced by an atom having the same atomic number but an atomic mass different from the atomic mass usually found in nature. Examples of isotopes that can be incorporated into compounds of the invention and pharmaceutically acceptable salts thereof include isotopes of hydrogen, carbon,

nitrogen, oxygen, phosphorus, sulphur, fluorine and chlorine such as ^{2}H, ^{3}H, ^{13}C, ^{14}C, ^{15}N, ^{17}O, ^{18}O, ^{31}P, ^{32}P, ^{35}S, ^{18}F and ^{36}Cl, respectively. Certain isotopic variations of the compounds of the invention and pharmaceutically acceptable salts thereof, for example, those in which a radioactive isotope such as ^{3}H or ^{14}C is incorporated, are useful in drug and/or substrate tissue distribution studies. Tritiated, i.e., ^{3}H, and carbon-14, i.e., ^{14}C, isotopes are particularly preferred for their ease of preparation and detectability. Further, substitution with isotopes such as deuterium, i.e., ^{2}H, may afford certain therapeutic advantages resulting from greater metabolic stability, for example, increased *in vivo* half-life or reduced dosage requirements and hence may be preferred in some circumstances. Isotopic variations in compounds of the invention and pharmaceutically acceptable salts thereof can generally be prepared by conventional procedures such as by the illustrative methods or by the preparations described in the Examples and Preparations hereafter using appropriate isotopic variations of suitable reagents.

It will be appreciated by those skilled in the art that certain protected derivatives of the compounds of the invention, which may be made prior to a final deprotection stage, may not possess pharmacological activity as such, but may, in certain instances, be administered orally or parenterally and thereafter metabolised in the body to form compounds of the invention which are pharmacologically active. Such derivatives may therefore be described as "prodrugs". Further, certain compounds of the invention may act as prodrugs of other compounds of the invention.

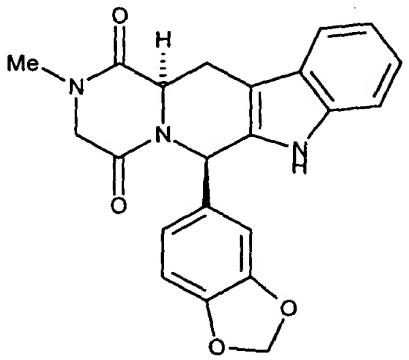
All protected derivatives, and prodrugs, of compounds of the invention are included within the scope of the invention. Examples of suitable pro-drugs for the compounds of the present invention are described in Drugs of Today, Volume 19, Number 9, 1983, pp 499 – 538 and in Topics in Chemistry, Chapter 31, pp 306 – 316 and in "Design of Prodrugs" by H. Bundgaard, Elsevier, 1985, Chapter 1 (the disclosures in which documents are incorporated herein by reference).

It will further be appreciated by those skilled in the art, that certain moieties, known to those skilled in the art as "pro-moieties", for example as described by H. Bundgaard in "Design of Prodrugs" (the disclosure in which document is incorporated herein by reference) may be placed on appropriate functionalities when such functionalities are present within compounds of the invention.

Preferred prodrugs for compounds of the invention include: esters, carbonate esters, hemi-esters, phosphate esters, nitro esters, sulfate esters, sulphoxides, amides, carbamates, azo-compounds, phosphamides, glycosides, ethers, acetals and ketals.

The compounds of the invention may also be combined with the following for the treatment of FSD (in particular FSAD):

Potentiators of cGMP (such as Sildenafil) and/or a centrally acting pharmaceutical (e.g. a dopamine agonist, such as apomorphine). Teachings on the use of apomorphine as a pharmaceutical may be found in US-A-5945117. In that particular document, apomorphine is delivered sub-lingually. In addition, or in the alternative, the agent may be used in combination with one or more of: one or more of a PDE inhibitor such as PDE2 (e.g. EHNA, and example 100 of EP 0771799-incorporated herein by reference) and such as a PDE5 inhibitor (eg sildenafil, 1-{[3-(3,4-dihydro-5-methyl-4-oxo-7-propylimidazo[5,1-f]-as-trazin-2-yl)-4-ethoxyphenyl]sulfonyl}-4-ethylpiperazine i.e. vardenafil / Bayer BA 38-9456) and IC351 (see structure below, Icos Lilly), one or more of a dopamine receptor agonist (eg apomorphine), one or more of a melanocortin receptor agonist (eg Melanotan II), one or more of a potassium channel opener (eg a KATP channel opener and/or a calcium activated potassium channel opener (eg minoxidil, nicorandil)), one or more of a hormone replacement therapy (eg HRT) agent, one or more of a testosterone replacement agent (inc DHEA (dehydroandrostendione)), one or more of an estrogen agonists , one or more of a serotonin receptor agonist, one or more of a prostinoid receptor agonist (eg alprostadil), one or more of an NPY (e.g. NPYY1) antagonist, one or more of a VIP agonist .



IC351 (Icos Lilly)

If a combination of active agents are administered, then they may be administered simultaneously, separately or sequentially.

The compounds of the invention, their pharmaceutically acceptable salts, and pharmaceutically acceptable solvates of either entity can be administered alone but, in human therapy will generally be administered in admixture with a suitable pharmaceutical excipient diluent or carrier selected with regard to the intended route of administration and standard pharmaceutical practice.

The compositions of the present invention may be administered by direct injection. The composition may be formulated for parenteral, mucosal, intramuscular, intravenous, subcutaneous, ocular, intraocular or transdermal administration. Depending upon the need, the agent may be administered at a dose of from 0.01 to 30 mg/kg body weight, such as from 0.1 to 10 mg/kg, more preferably from 0.1 to 1 mg/kg body weight.

The term "administered" includes delivery by viral or non-viral techniques. Viral delivery mechanisms include but are not limited to adenoviral vectors, adeno-associated viral (AAV) vectos, herpes viral vectors, retroviral vectors, lentiviral vectors, and baculoviral vectors. Non-viral delivery mechanisms include lipid mediated transfection, liposomes, immunoliposomes, lipofectin, cationic facial amphiphiles (CFAs) and combinations thereof. The routes for such delivery mechanisms include but are not limited to mucosal, nasal, oral, parenteral, gastrointestinal, topical, or sublingual routes.

In addition or in the alternative the compositions (or component parts thereof) of the present invention may be administered by direct injection. In addition or in the alternative the compositions (or component parts thereof) of the present invention may be administered topically. In addition or in the alternative the compositions (or component parts thereof) of the present invention may be administered by inhalation. In addition or in the alternative the compositions (or component parts thereof) of the present invention may also be administered by one or more of: a mucosal route, for example, as a nasal spray or aerosol for inhalation or as an ingestable solution such as by an oral route, or by a parenteral route where delivery is by an injectable form, such as, for example, by a rectal, ophthalmic (including intravitreal or intracameral), nasal, topical (including buccal and sublingual), intrauterine, vaginal or parenteral (including subcutaneous, intraperitoneal, intramuscular, intravenous, intradermal,

intracranial, intratracheal, and epidural) transdermal, intraperitoneal, intracranial, intracerebroventricular, intracerebral, intravaginal, intrauterine, or parenteral (e.g., intravenous, intraspinal, subcutaneous, transdermal or intramuscular) route.

By way of further example, the pharmaceutical composition of the present invention may be administered in accordance with a regimen of 1 to 10 times per day, such as once or twice per day. The specific dose level and frequency of dosage for any particular patient may be varied and will depend upon a variety of factors including the activity of the specific compound employed, the metabolic stability and length of action of that compound, the age, body weight, general health, sex, diet, mode and time of administration, rate of excretion, drug combination, the severity of the particular condition, and the individual undergoing therapy.

Hence, the term "administered" includes but is not limited to delivery by a mucosal route, for example, as a nasal spray or aerosol for inhalation or as an ingestable solution; a parenteral route where delivery is by an injectable form, such as, for example, an intravenous, intramuscular or subcutaneous route.

For example, the compounds of the invention or salts or solvates thereof can be administered orally, buccally or sublingually in the form of tablets, capsules, ovules, elixirs, solutions or suspensions, which may contain flavouring or colouring agents, for immediate-, delayed-, modified-, or controlled-release applications. The compounds of the invention may also be administered via intracavernosal injection.

Such tablets may contain excipients such as microcrystalline cellulose, lactose, sodium citrate, calcium carbonate, dibasic calcium phosphate and glycine, disintegrants such as starch (preferably corn, potato or tapioca starch), sodium starch glycollate, croscarmellose sodium and certain complex silicates, and granulation binders such as polyvinylpyrrolidone, hydroxypropylmethyl cellulose (HPMC), hydroxypropylcellulose (HPC), sucrose, gelatin and acacia. Additionally, lubricating agents such as magnesium stearate, stearic acid, glyceryl behenate and talc may be included.

Solid compositions of a similar type may also be employed as fillers in gelatin capsules. Preferred excipients in this regard include lactose, starch, a cellulose, milk

sugar or high molecular weight polyethylene glycols. For aqueous suspensions and/or elixirs, the compounds of the invention may be combined with various sweetening or flavouring agents, colouring matter or dyes, with emulsifying and/or suspending agents and with diluents such as water, ethanol, propylene glycol and glycerin, and combinations thereof.

The compounds of the invention can also be administered parenterally, for example, intracavernosally, intravenously, intra-arterially, intraperitoneally, intrathecally, intraventricularly, intraurethrally intrasternally, intracranially, intramuscularly or subcutaneously, or they may be administered by infusion techniques. For such parenteral administration they are best used in the form of a sterile aqueous solution which may contain other substances, for example, enough salts or glucose to make the solution isotonic with blood. The aqueous solutions should be suitably buffered (preferably to a pH of from 3 to 9), if necessary. The preparation of suitable parenteral formulations under sterile conditions is readily accomplished by standard pharmaceutical techniques well-known to those skilled in the art.

For oral and parenteral administration to human patients, the daily dosage level of the compounds of the invention or salts or solvates thereof will usually be from 10 to 1000 mg (in single or divided doses).

Thus, for example, tablets or capsules of the compounds of the invention or salts or solvates thereof may contain from 5 to 1000mg, such as 5 mg to 500 mg of active compound for administration singly or two or more at a time, as appropriate. The physician in any event will determine the actual dosage which will be most suitable for any individual patient and it will vary with the age, weight and response of the particular patient. The above dosages are exemplary of the average case. There can, of course, be individual instances where higher or lower dosage ranges are merited and such are within the scope of this invention. The skilled person will also appreciate that, in the treatment of certain conditions (including MED and FSD), compounds of the invention may be taken as a single dose on an "as required" basis (i.e. as needed or desired).

The compounds of the invention can also be administered intranasally or by inhalation and are conveniently delivered in the form of a dry powder inhaler or an aerosol spray

presentation from a pressurised container, pump, spray or nebuliser with the use of a suitable propellant, e.g. dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, a hydrofluoroalkane such as 1,1,1,2-tetrafluoroethane (HFA 134A [trade mark] or 1,1,1,2,3,3,3-heptafluoropropane (HFA 227EA [trade mark]), carbon dioxide or other suitable gas. In the case of a pressurised aerosol, the dosage unit may be determined by providing a valve to deliver a metered amount. The pressurised container, pump, spray or nebuliser may contain a solution or suspension of the active compound, e.g. using a mixture of ethanol and the propellant as the solvent, which may additionally contain a lubricant, e.g. sorbitan trioleate. Capsules and cartridges (made, for example, from gelatin) for use in an inhaler or insufflator may be formulated to contain a powder mix of a compound of the invention and a suitable powder base such as lactose or starch.

Aerosol or dry powder formulations are preferably arranged so that each metered dose or "puff" contains from 1 to 50 mg of a compound of the invention for delivery to the patient. The overall daily dose with an aerosol will be in the range of from 1 to 50 mg which may be administered in a single dose or, more usually, in divided doses throughout the day.

Alternatively, the compounds of the invention or salts or solvates thereof can be administered in the form of a suppository or pessary, or they may be applied topically in the form of a gel, hydrogel, lotion, solution, cream, ointment or dusting powder. The compounds of the invention or salts or solvates thereof may also be dermally administered. The compounds of the invention or salts or solvates thereof may also be transdermally administered, for example, by the use of a skin patch. They may also be administered by the ocular, pulmonary or rectal routes.

For ophthalmic use, the compounds can be formulated as micronised suspensions in isotonic, pH adjusted, sterile saline, or, preferably, as solutions in isotonic, pH adjusted, sterile saline, optionally in combination with a preservative such as a benzylalkonium chloride. Alternatively, they may be formulated in an ointment such as petrolatum.

For application topically to the skin, the compounds of the invention or salts or solvates thereof can be formulated as a suitable ointment containing the active compound

suspended or dissolved in, for example, a mixture with one or more of the following: mineral oil, liquid petrolatum, white petrolatum, propylene glycol, polyoxyethylene polyoxypropylene compound, emulsifying wax and water. Alternatively, they can be formulated as a suitable lotion or cream, suspended or dissolved in, for example, a mixture of one or more of the following: mineral oil, sorbitan monostearate, a polyethylene glycol, liquid paraffin, polysorbate 60, cetyl esters wax, cetearyl alcohol, 2-octyldodecanol, benzyl alcohol and water.

The compounds of the invention may also be used in combination with a cyclodextrin. Cyclodextrins are known to form inclusion and non-inclusion complexes with drug molecules. Formation of a drug-cyclodextrin complex may modify the solubility, dissolution rate, bioavailability and/or stability property of a drug molecule. Drug-cyclodextrin complexes are generally useful for most dosage forms and administration routes. As an alternative to direct complexation with the drug the cyclodextrin may be used as an auxiliary additive, e.g. as a carrier, diluent or solubiliser. Alpha-, beta- and gamma-cyclodextrins are most commonly used and suitable examples are described in WO-A-91/11172, WO-A-94/02518 and WO-A-98/55148.

In a preferred embodiment, the compounds of the invention are delivered systemically (such as orally, buccally, sublingually), more preferably orally. Preferably such systemic (most preferably oral) administration is used to treat sexual dysfunction in a male or female, more preferably FSD and more preferably still FSAD.

Thus in a particularly preferred embodiment, there is provided the use of the compounds of the invention in the manufacture of a systemically delivered (preferably orally delivered) medicament for the treatment or prophylaxis of FSD, more preferably FSAD.

Since NEP is present throughout the body, it is very unexpected that the compounds of the invention can be administered systemically and achieve a therapeutic response in the genitalia (preferably the female genitalia) without provoking intolerable (adverse) side effects. Thus in the in vivo (rabbit) results hereafter, the compounds of the invention administered systemically increased genital blood flow, upon sexual arousal (mimiced by pelvic nerve stimulation) without adversely affecting cardiovascular parameters, such as causing a significant hypotensive or hypertensive.

Preferably the compounds of the invention are administered for the treatment of sexual dysfunction (more preferably FSD) in the sexually stimulated patient (by sexual stimulation we mean to include visual, auditory or tactile stimulation).The stimulation can be before, after or during said administration.

Thus the compounds of the invention enhance the pathways/mechanisms that underlie sexual aroual in the female gentialia restoring or improving the sexual arousal response to sexual stimulation.

Thus a preferred embodiment provides the use of a compound of the invention in the preparation of a medicament for the treatment or prophyaxis of sexual dysfunction (more preferably FSD) in the stimulated patient.

For veterinary use, a compound of the invention or a veterinarily acceptable salt thereof, or a veterinarily acceptable solvate or pro-drug thereof, is administered as a suitably acceptable formulation in accordance with normal veterinary practice and the veterinary surgeon will determine the dosing regimen and route of administration which will be most appropriate for a particular animal.

Compounds of the invention may be prepared, in known manner, in a variety of ways.

Throughout the specification, general formulae are designated by Roman numerals I, II, III, IV etc. Subsets of these general formulae are defined as Ia, Ib, Ic etc, ... IVa, IVb, IVc etc.

According to a further aspect of the invention, compounds of general formula I may be prepared according to reaction scheme 1, by reacting a compound of formula II (where Prot is a suitable protecting group) with a primary amine of formula III to give a compound of formula IV. Deprotection gives compounds of formula I.

Compounds of formula II and III are novel and form a further aspect of the invention.

Preferred reaction conditions for the acid/amine coupling step comprise reacting II with III (or its amine salt) in the presence of an activating agent, optionally a catalyst, and an excess of an acid acceptor, in a suitable solvent. Particularly preferred reaction

conditions comprise reacting II (1-1.5 equivalents), III (or its salt 1-1.5 equivalents), in the presence of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (WSCDI) or N,N'-dicyclohexylcarbodiimide (DCC) (1.1-1.3 equivalents), 1-hydroxybenzotrazole hydrate (HOBT) or dimethylaminopyridine (DMAP) (1.05-1.2 equivalents), *N*-methyl morpholine (NMM) or triethyamine (2.3-3 equivalents), in dimethylformamide or dichloromethane at between room temperature and 90°C for 16-18 hours.

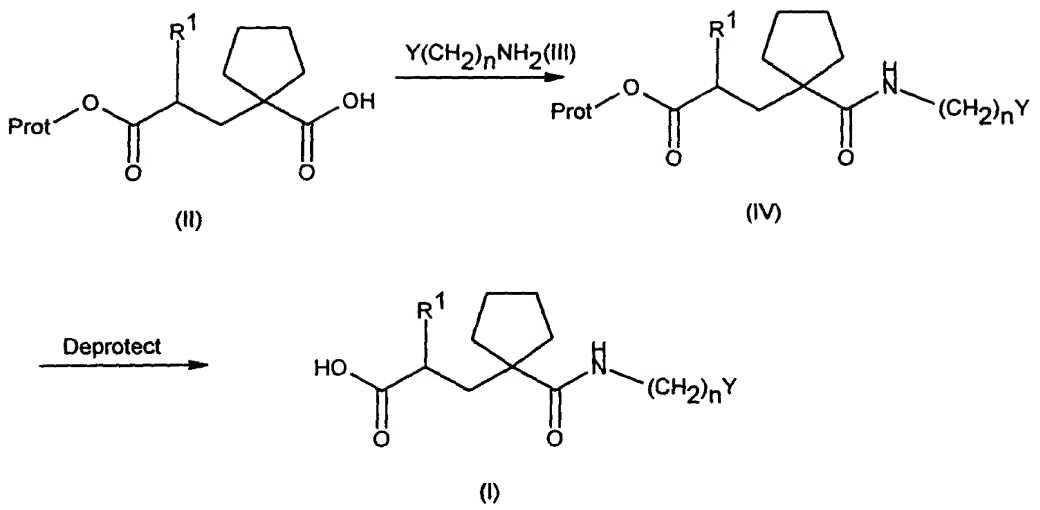
Alternatively, the acid/amine coupling step may be prepared via the acid chloride in the presence of an excess of acid acceptor, in a suitable solvent. The acid chloride may be isolated or it may be generated in situ. Preferred reaction conditions comprise reacting the acid chloride of II (1-1.1 equivalents), III (or its salt, 1 to 1.5 equivalents), triethyamine or *N*-methyl morpholine (1.4-10 equivalents), in dichloromethane at room temperature for 24 hours. Compounds of formula II can be converted to the acid chloride *in situ* by treatment with oxalyl chloride in dichloromethane in the presence of a catalytic amount of dimethylformamide for 2 hours at room temperature.

Methods for deprotection of an acid group depend on the protecting group. For examples of protection/deprotection methodology see "Protective groups in Organic synthesis", TW Greene and PGM Wutz.

For example, when Prot is a *tert*-butyl, deprotection conditions comprise reacting IV with trifluoroacetic acid/dichloromethane (1:1-1.5 by volume), at room temperature for 2-18 hours, optionally in the presence of a carbocation scavenger, e.g. anisole (10 equivalents). When Y contains a hydroxy group, base hydrolysis of the intermediate trifluoroacetic acid ester may be necessary. Alternative methodology for deprotection when Prot is *tert*-butyl comprises treating II with hydrochloric acid in dichloromethane at room temperature for 3 hours. For the avoidance of doubt, Prot as *tert*-butyl is given by way of Example and is not intended to be limited to *tert*-Butyl.

When Prot is benzyl, deprotection conditions comprise reacting IV with palladium on charcoal (5-10%) in aqueous ethanol (40-95%) at 15-60 psi at room temperature for 2hrs to 3 days.

Scheme 1

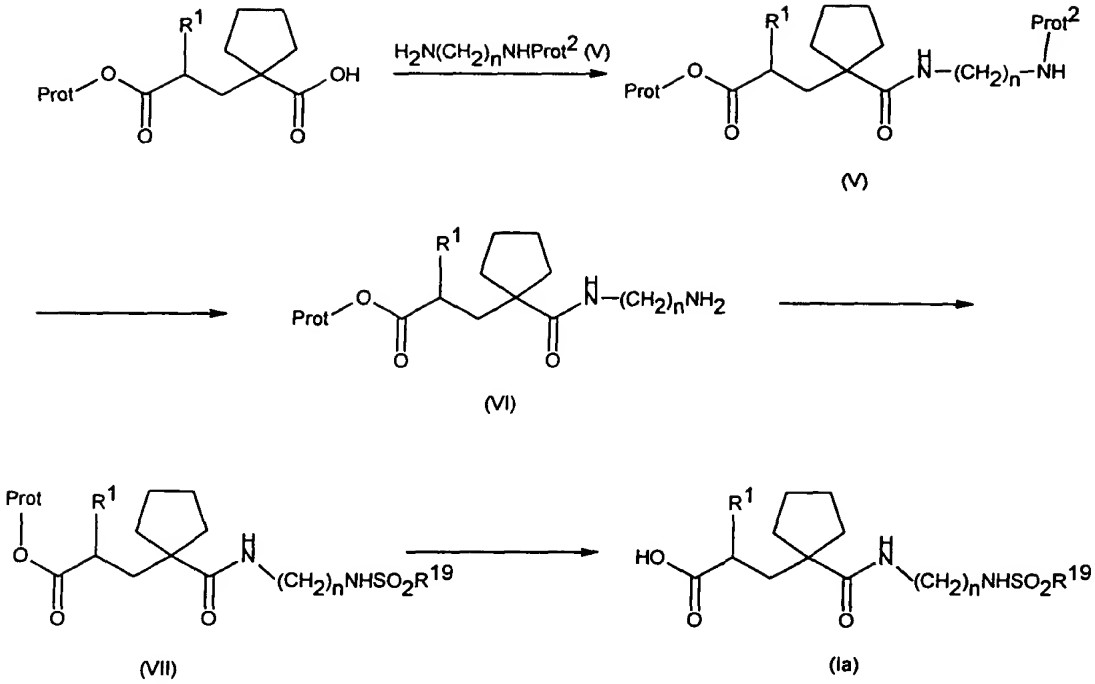


Compounds of formula Ia, i.e. compounds of general formula I where Y is - $NHSO_2R^{19}$, may be prepared according to reaction scheme 2. Compounds of formula V are first prepared by reacting compounds of formula II with compounds of formula V where $Prot^2$ is a suitable amine protecting group. Preferred reaction conditions are analogous to those described the acid/amine coupling step for Scheme 1 above. Selective amine deprotection of compounds of formula V gives compounds of formula VI. Compounds of formula VI are reacted with $R^{19}SO_2Cl$ in the presence of an acid acceptor in a suitable solvent to form compounds of formula VII. Deprotection of compounds of formula VII under analogous conditions to those described for the deprotection step of Scheme 1 gives compounds of formula Ia.

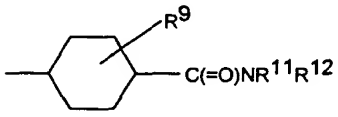
Methods for deprotection of an amine group depend on the protecting group. For examples of protection/deprotection methodology see "Protective groups in Organic Synthesis", TW Greene and PGM Wutz. For example, when $Prot^2$ is benzoyloxycarbonyl, deprotection conditions comprise reacting V with palladium on charcoal (10%) in ethanol at room temperature for 18 hours.

Preferred methods for preparation of the compounds of formula VII comprise reaction of VI with $R^{19}SO_2Cl$ (1 equivalent) in the presence of triethyamine (1.5-2.5 equivalents) in dichloromethane at room temperature for 2 to 3 days.

Scheme 2



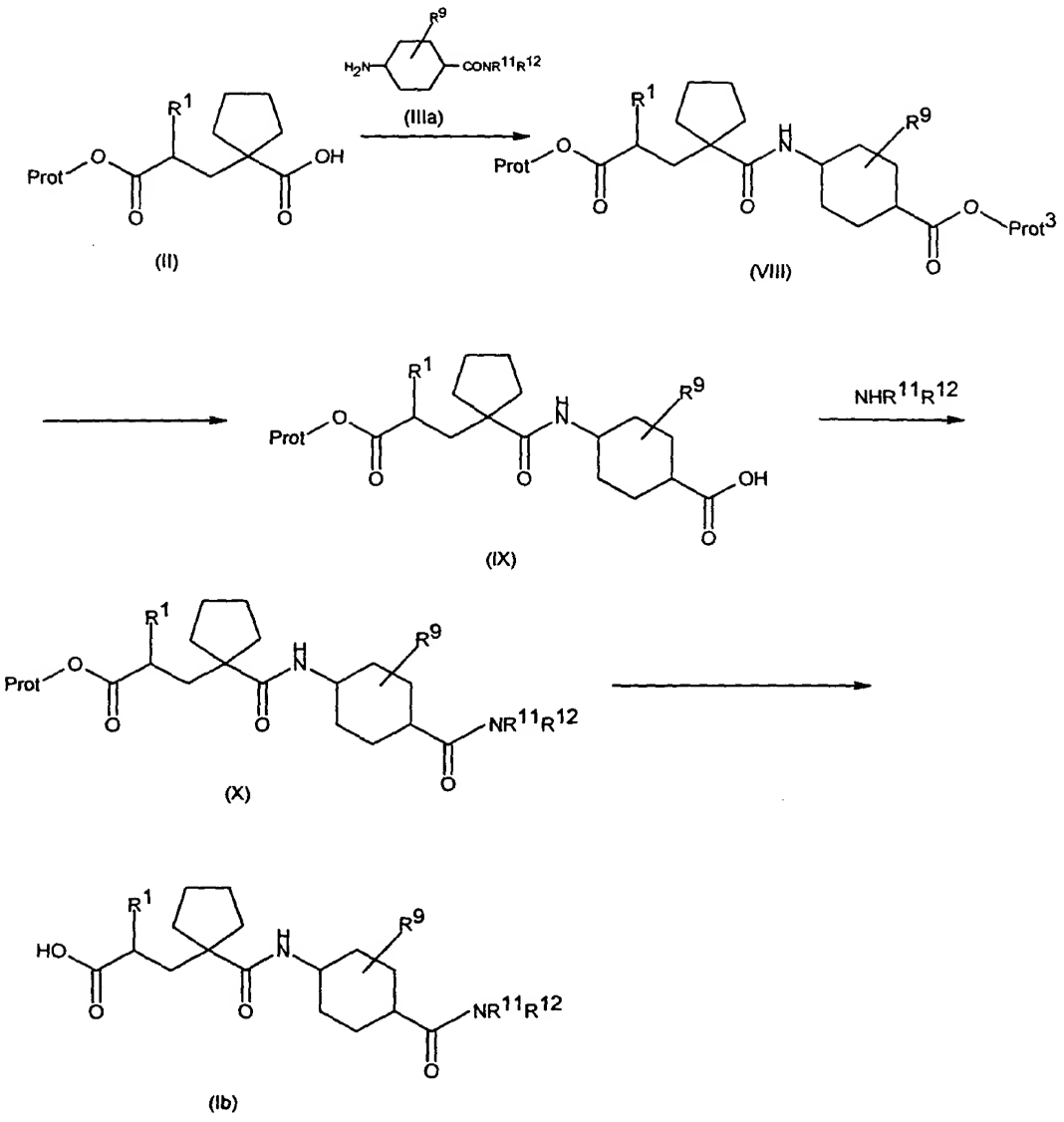
Compounds of formula Ib, i.e. compounds of formula I where n is 0 and Y is



, may be prepared according to reaction scheme 3. Compounds of formula II are reacted with compounds of formula IIIa under analogous conditions to acid/amine coupling conditions of Scheme 1 to give compounds of formula VIII, where $Prot^3$ is a protecting group which can be selectively removed in the presence of protecting group Prot. A preferred protecting group $Prot^3$ is a base labile ester group. Consequently, treatment of compound of formula VIII under basic conditions gives compounds of formula IX. Compounds of formula IX are reacted with compounds of formula $NHR^{11}R^{12}$ under analogous conditions to acid/amine coupling conditions of Scheme 1 to form compounds of formula X. Deprotection of compounds of formula X under analogous conditions to the deprotection step in Scheme 1 gives compounds of formula Ib.

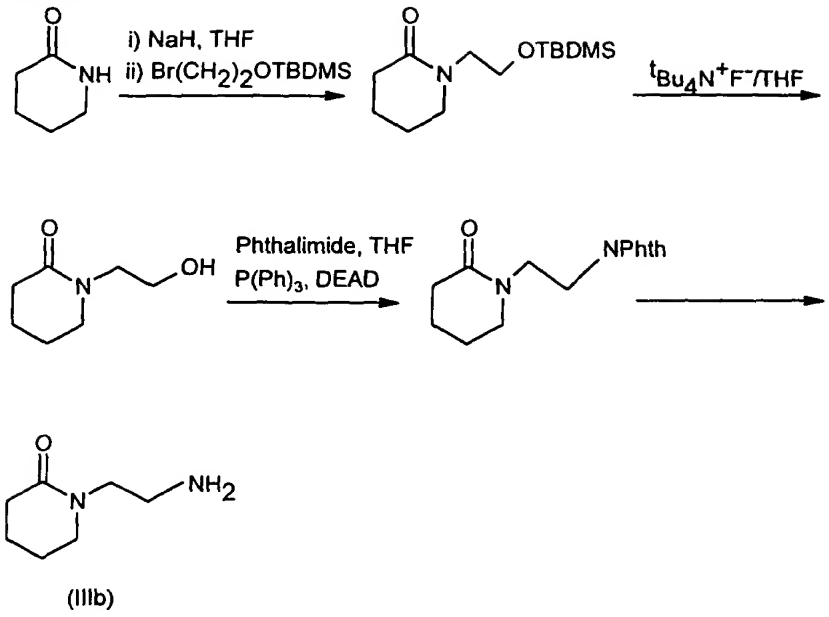
Preferred conditions for removal of protecting group $Prot^3$ from VIII comprise treatment of VIII with sodium hydroxide (1N) in methanol at room temperature for 22 hours.

Scheme 3



Compounds of formula IIIb, i.e. compounds of general formula III where n is 2, Y is 2-oxopiperidino, may be prepared according to reaction scheme 4.

Scheme 4



(IIIb)

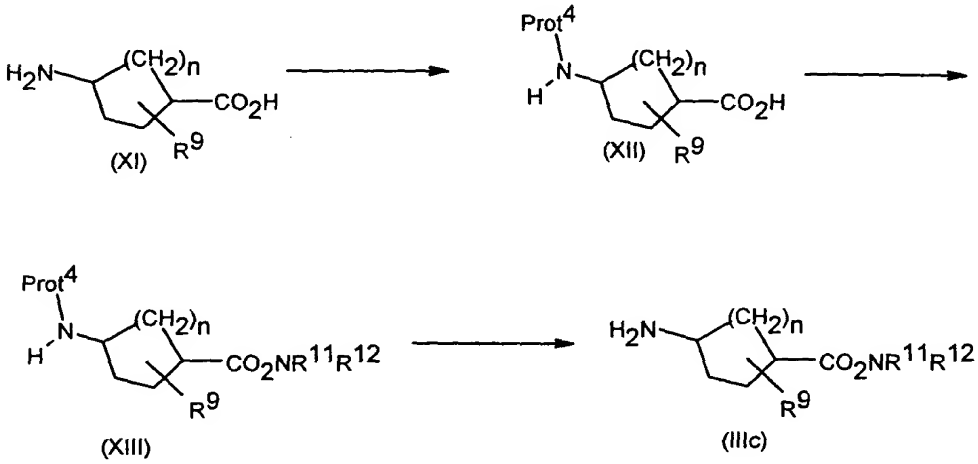
Compounds of formula IIIc where n is 1 or 2, may be prepared according to reaction scheme 5. Compounds of formula XI are protected at the amine moiety with a suitable protecting group $Prot^4$ to form compounds of formula XII. A preferred protecting group is *tert*-butyloxycarbonyl. Compounds of formula XII are reacted under typical acid/amine coupling conditions with $NHR^{11}R^{12}$ to form compounds of formula XIII, which on deprotection form compounds of formula IIIb.

Typical reaction conditions for introducing the *tert*-butyloxycarbonyl protecting group comprise treating XI with (*tert*-butyloxycarbonyl)$_2$O in dioxan and 2N sodium hydroxide at room temperature for 18 hrs.

Typical acid/amine coupling conditions comprise treating XII and $NHR^{11}R^{12}$ with benzotriazol-1-yloxytris(pyrrolidino)phosphonium hexafluorophosphate (PYBOP), 1-hydroxybenzotrazole hydrate (HOBT), Hünigs base, an amine (eg triethylamine), in dimethylformamide at room temperature for 2hrs. Alternatively, XII and $NHR^{11}R^{12}$ may be treated with1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride, HOBT, N-methyl morpholine (NMM), in dimethylformamide at room temperature for 18 hrs.

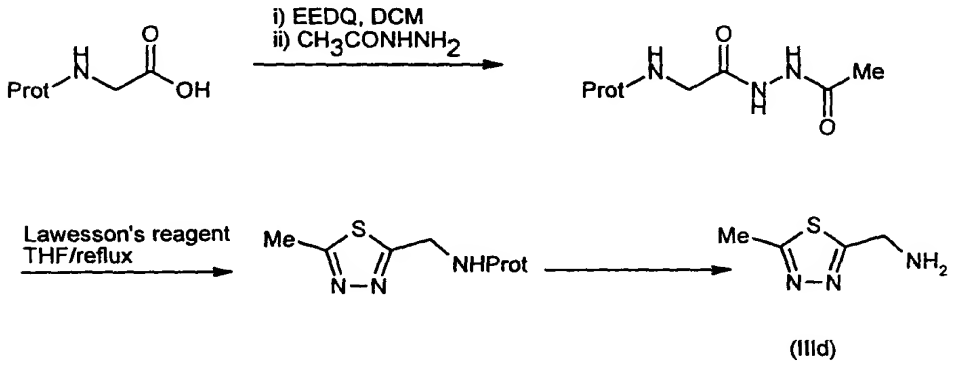
Typical reaction conditions for deprotection when $Prot^4$ is *tert*-butyloxycarbonyl comprise reacting XIII with hydrochloric acid or trifluoroacetic acid in dichloromethane at room temperature for 2 to 4 hrs

Scheme 5



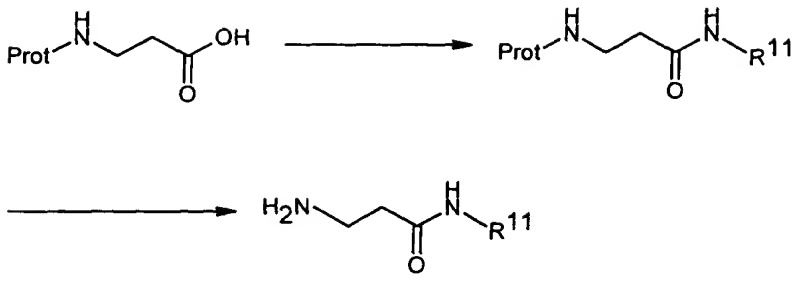
Compounds of formula IIId can be prepared according to reaction scheme 6. The protecting group is preferably *tert*-butyloxycarbonyl, which is removed under standard conditions, as previously described.

Scheme 6



Compounds of formula IIIe are prepared according to reaction scheme 7 using standard acid/amine coupling reactions, as previously described. The protecting group is preferably benzyloxycarbonyl which may be removed under standard conditions, typically palladium on charcoal (5-10%) in ethanol at room temperature and 50 psi for 4 hrs.

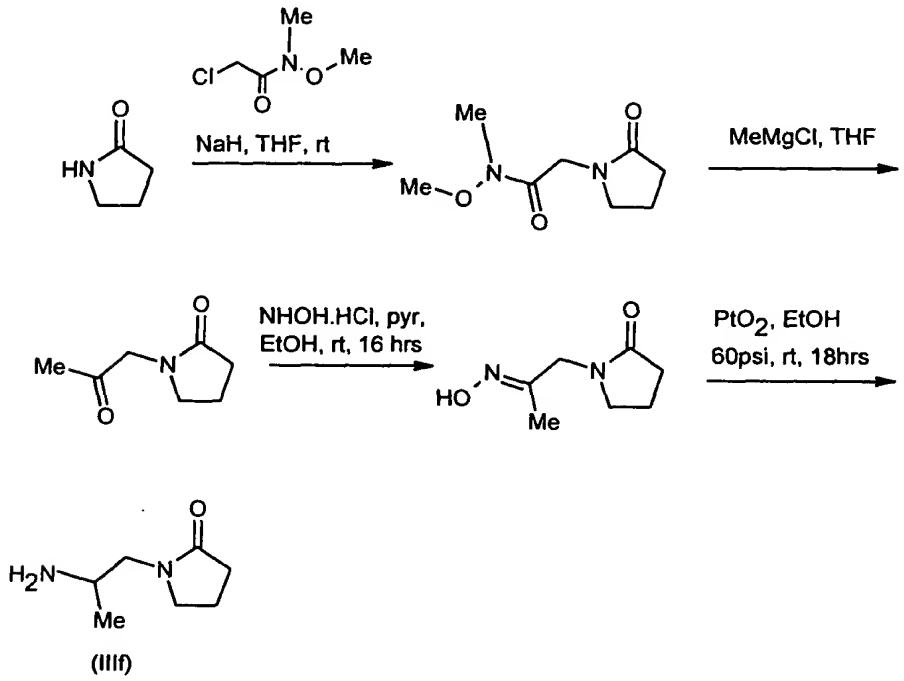
Scheme 7



(IIIe)

Compounds of formula IIIf may be prepared according to reaction scheme 8.

Scheme 8

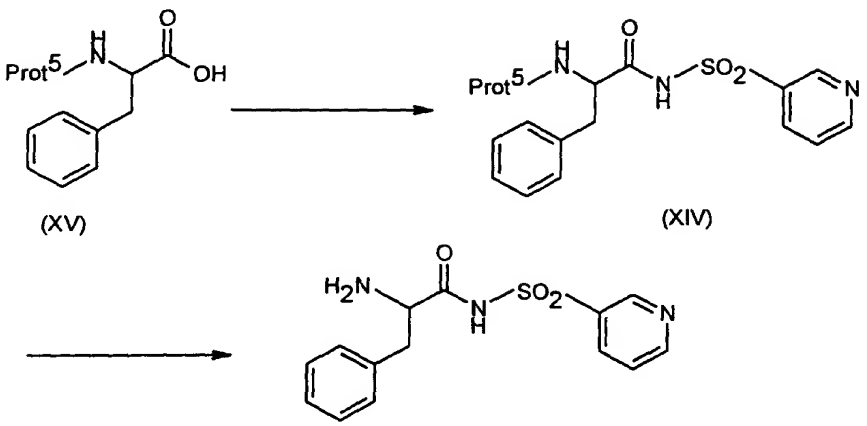


(IIIf)

Compounds of formula IIIg may be prepared in two steps according to reaction scheme 9. As a first step, compounds of formula XIV are prepared from compounds of formula XV using standard acid/amine coupling methodology analogous to the acid/amine coupling conditions described for reaction scheme 1. Prot^5 represents a suitable leaving group, preferably *tert*-butyloxycarbonyl. The second step comprises removal of Prot^5. When Prot^5 is *tert*-butyloxycarbonyl then preferred reaction

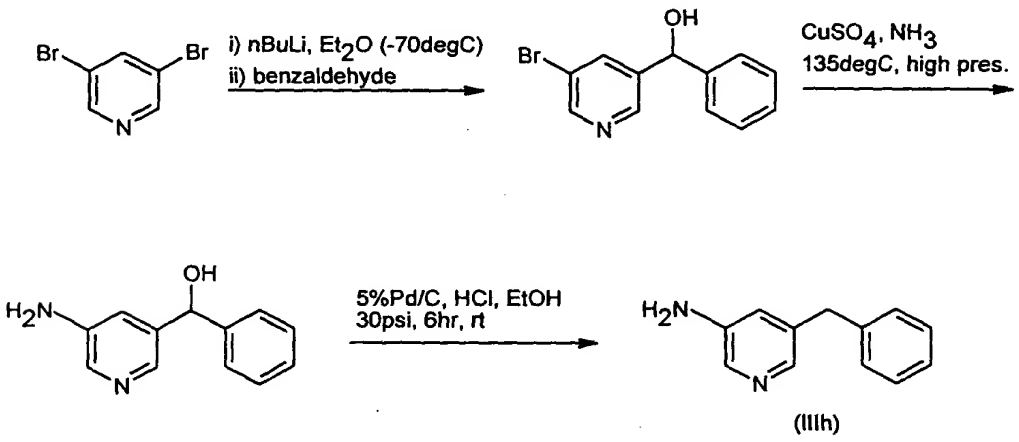
conditions comprise treatment with hydrochloric acid in diethyl ether/ethyl acetate at room temperature for 18 hrs.

Scheme 9



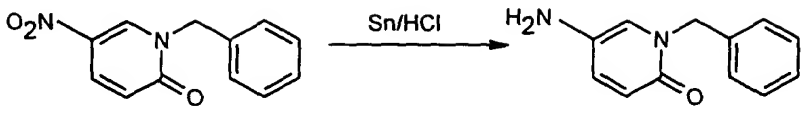
Compounds of formula IIIh may be prepared in three steps according to reaction scheme 10.

Scheme 10



Compounds of formula IIIj may be prepared by reduction of a nitro group according to reaction scheme 11.

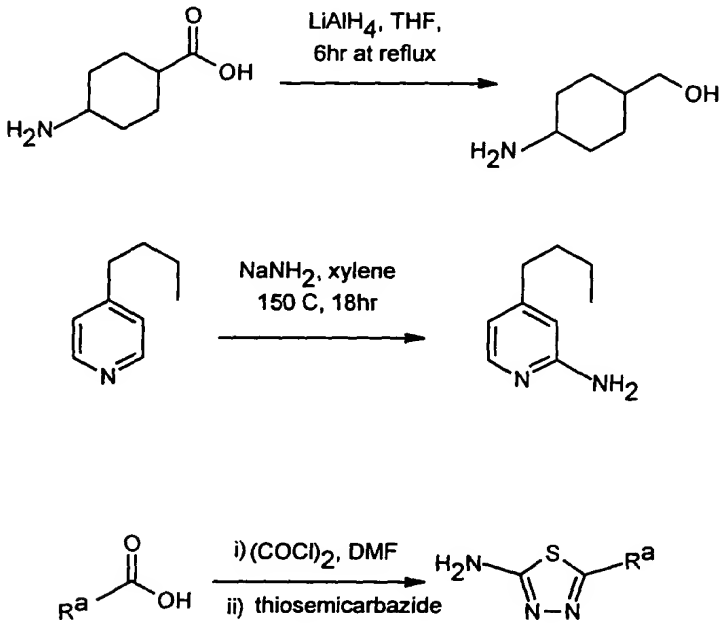
Scheme 11



(IIIj)

Further methods for preparing compounds of formula III are give in Scheme 12 below, where R^a is C_{1-6}alkyl or alkoxy.

Scheme 12



All of the above reactions and the preparations of novel starting materials used in the preceding methods are conventional and appropriate reagents and reaction conditions for their performance or preparation as well as procedures for isolating the desired products will be well-known to those skilled in the art with reference to literature precedents and the Examples and Preparations hereto.

A pharmaceutically acceptable salt of a compound of the formula (I) may be readily prepared by mixing together solutions of a compound of the formula (I) and the desired acid or base, as appropriate. The salt may precipitate from solution and be collected by filtration or may be recovered by evaporation of the solvent.

It is to be appreciated that all references herein to treatment include curative, palliative and prophylactic treatment.

The following formulation examples are illustrative only and are not intended to limit the scope of the invention . "Active ingredient" means a compound according to formula 1 or a pharmaceutically acceptable salt thereof.

Formulation 1: A tablet is prepared using the following ingredients:

	weight/mg
Active ingredient	250
Cellulose, microcrystalline	400
Silicon dioxide, fumed	10
Stearic acid	5
Total	665

the components are blended and compressed to form tablets each weighing 665mg.

Formulation 2: An intravenous formulation may be prepared as follows:

Active ingredient	100mg
Isotonic saline	1,000ml

The invention additionally includes:

(i) a pharmaceutical composition including a compound of the second aspect of the invention, together with a pharmaceutically acceptable excipient, diluent or carrier;

(ii) a compound of the second aspect of the invention for use as a medicament;

(iii) a method of treating sexual dysfunction (preferably FSD) in a mammal including treating said mammal with an effective amount of a compound of the invention;

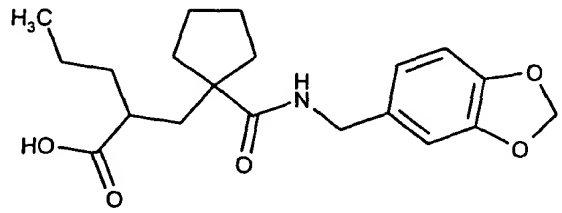
(iv) A sexual dysfunction treating pharmaceutical composition comprising a compound of the invention together with a pharmaceutically acceptable excipient, diluent or carrier.

The following Examples illustrate the preparation of the compounds of general formula (I):

Examples

Example 1

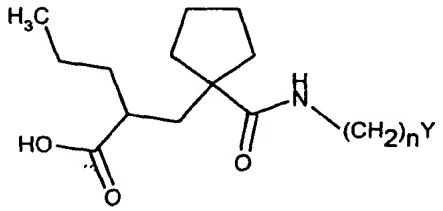
2-({1-[(1,3-Benzodioxol-5-ylamino)carbonyl]cyclopentyl}methyl)pentanoic acid



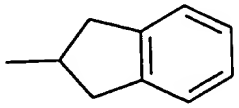
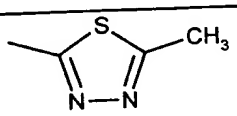
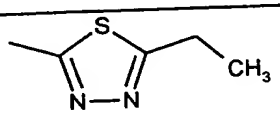
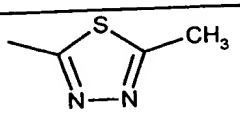
Trifluoroacetic acid (5ml) was added to a solution of the *tert*-butyl ester from preparation 34 (130mg, 0.31mmol) in dichloromethane (5ml), and the solution stirred at room temperature for 4 hours. The reaction mixture was concentrated under reduced pressure and the residue azeotroped with toluene and dichloromethane to afford the title compound as a clear oil, 112 mg, ^{1}H NMR ($CDCl_3$, 400MHz) δ 0.83 (t, 3H), 1.22-1.40 (m, 3H), 1.50-1.72 (m, 8H), 1.95 (m, 1H), 2.10 (m, 2H), 2.19 (m, 1H), 4.30 (m, 2H), 5.93 (s, 2H), 5.99 (bs, 1H), 6.74 (m, 3H); LRMS: m/z 380 (MH^-).

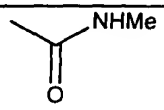
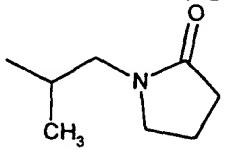
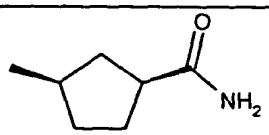
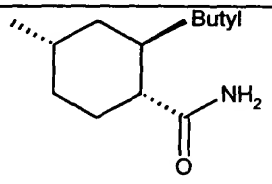
Examples 2 to 9

Compounds of formula Ic, I.e. Compounds of general formula I where R^1 is propyl, where prepared from the corresponding *tert*-butyl ester, following a similar procedure to that described in Example 1.



(Ic)

Ex	n	R	Yield	Data
2[1]	0		78	^{1}H NMR ($CDCl_3$, 400MHz) δ: 0.81 (s, 3H), 1.17-2.04 (m, 14H), 2.27-2.38 (m, 1H), 2.64-2.80 (m, 2H), 3.20-3.31 (m, 2H), 4.60-4.72 (m, 1H), 5.97 (d, 1H), 7.03-7.18 (m, 4H). LRMS : m/z 343.8 (M^+).
3[2,3]	0		81	^{1}H NMR ($CDCl_3$, 400MHz) δ: 0.90 (t, 3H), 1.30-1.42 (m, 4H), 1.59-1.81 (m, 7H), 2.18 (m, 1H), 2.30 (m, 1H), 2.42 (m, 1H), 2.55 (m, 1H), 2.61 (s, 3H). LRMS : m/z 324 (MH^-). Mp 184-186°C Anal. Found: C, 55.50; H, 7.22; N, 12.61. $C_{15}H_{23}N_3O_3S$ requires C, 55.36; H, 7.14; N, 12.91%.
4[3]	0		86	^{1}H NMR ($CDCl_3$, 400MHz) δ: 0.92 (t, 3H), 1.35 (t, 3H), 1.25-1.80 (m, 11H), 2.20-2.50 (m, 4H), 2.95 (q, 2H), 12.10 (bs, 1H). LRMS : m/z 339.8 (MH^+) Anal. Found: C, 56.46; H, 7.46; N, 12.36. $C_{16}H_{25}N_3O_3S$ requires C, 56.62; H, 7.44; N, 12.37%.
5[2]	1		81	^{1}H NMR ($CDCl_3$, 400MHz) δ: 0.80 (t, 3H), 1.20-1.70 (m, 11H), 1.90-2.20 (m, 3H), 2.25 (m, 1H), 2.70 (s, 3H), 4.75 (m, 2H), 7.10 (bs, 1H). LRMS : m/z 340.6 (MH^+)

Ex	n	R	Yield	Data
6[2]	2		45	^{1}H NMR ($CDCl_3$, 400MHz) δ: 0.88 (t, 3H), 1.25-1.40 (m, 3H), 1.41-1.70 (m, 8H), 1.92 (m, 1H), 2.00-2.18 (m, 2H), 2.38 (m, 1H), 2.42 (t, 2H), 2.80 (d, 3H), 3.40-3.60 (m, 2H), 6.50 (bs, 1H), 6.74 (bs, 1H). LRMS : m/z 313.2 (MH^+)
7	0		93	^{1}H NMR ($CDCl_3$, 400MHz) δ: 0.85 (t, 3H), 1.19 (d, 3H), 1.21-1.69 (m, 11H), 1.89-2.10 (m, 5H), 2.30 (m, 1H), 2.41 (m, 2H), 2.95 (m, 1H), 3.35 (m, 1H), 3.63 (m, 2H), 4.20 (m, 1H), 6.58-6.70 (m, 1H). LRMS : m/z 353.1 (MH^+)
8	0		99	^{1}H NMR ($CDCl_3$, 400MHz) δ: 0.81 (t, 3H), 1.20-1.39 (m, 3H), 1.41-2.10 (m, 1H), 2.80 (m, 1H), 4.35 (m, 17H), 5.81 (d, 1H), 6.30 (bs, 0.5H), 6.43 (bs, 0.5H), 7.40 (bd, 0.5H), 7.61 (bd, 0.5H). LRMS : m/z 339.8 (MH^+)
9	0			^{1}H NMR ($CDCl_3$, 400MHz) δ: 0.84 (m, 6H), 1.08-2.08 (m, 29H), 4.29 (m, 1H), 5.95 (d, 1H), 6.43 (s, 1H), 7.80 (d, 1H). LRMS : m/z 409.5 (MH^+)

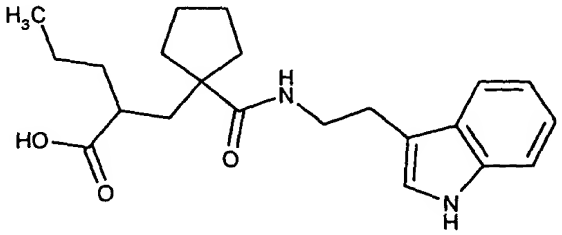
1 = additionally purified by column chromatography on silica gel using ethyl acetate:pentane as eluant.

2 = additionally purified by column chromatography on silica gel using dichloromethane:methanol as eluant.

3 = recrystallised from ther

Example 10

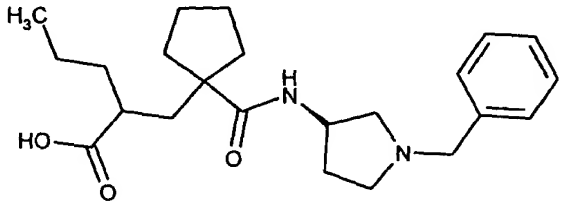
2-{[1-({[2-(1*H*-Indol-3-yl)ethyl]amino}carbonyl)cyclopentyl]methyl}pentanoic acid



Trifluoroacetic acid (2.61ml, 33.9mmol) was added to a solution of the *tert*-butyl ester from preparation 44 (482mg, 1.13mmol) and anisole (1.23ml, 11.3mmol) in dichloromethane (4ml), and the reaction stirred at room temperature for 4 hours. The mixture was washed with water, then brine, dried ($MgSO_4$), concentrated under reduced pressure and the residue azeotroped with toluene. The residual brown oil was purified by column chromatography on silica gel using dichloromethane:methanol (95:5) as eluant, and re-columned using an elution gradient of ethyl acetate:pentane (30:70 to 50:50) to afford the title compound as a clear foam, 136mg, 32%; ^{1}HNMR ($CDCl_3$, 400MHz) δ: 0.82 (s, 3H), 1.16-1.77 (m, 12H), 1.78-2.03 (m, 2H), 2.36 (m, 1H), 2.97 (m, 2H), 3.61 (m, 2H), 5.83 (m, 1H), 7.04 (s, 1H), 7.09-7.23 (m, 2H), 7.39 (d, 1H), 7.61 (d, 1H), 8.15 (m, 1H); LRMS : m/z 371.8 (MH^+.

Example 11

2-{[1-({[(3*S*)-1-Benzylpyrrolidinyl]amino}carbonyl)cyclopentyl]methyl}pentanoic acid

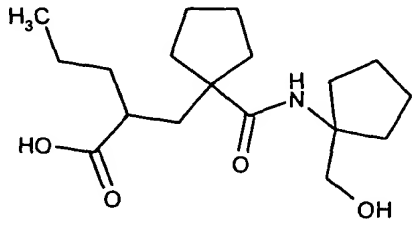


A solution of the *tert*-butyl ester from preparation 45 (70mg, 0.16mmol) in trifluoroacetic acid (1ml) and dichloromethane (1ml) was stirred at room temperature for 2 hours. The reaction was concentrated under reduced pressure and the residue azeotroped with dichloromethane. The residue was partitioned between water (1ml) and ethyl acetate (5ml), and the pH of the aqueous layer adjusted to 6 using sodium bicarbonate solution. The layers were separated, the organic phase dried (Na_2SO_4), evaporated under reduced pressure and the residue azeotroped with dichloromethane, to give the title compound as a beige foam, 45mg, 73%; ^{1}H NMR ($CDCl_3$, 400MHz) δ: 0.84 (t, 3H), 1.20-2.95 (m, 19H), 3.52 (m, 1H), 3.75 (m, 1H), 3.95 (m, 1H), 4.25 (m,

1H), 4.45 (m, 1H), 6.96 (bs, 1H), 7.39 (m, 5H); LRMS : m/z 387 (MH^+); Anal. Found: C, 61.11; H, 7.69; N, 6.00. $C_{23}H_{34}N_2O_3;CH_2Cl_2$ requires C, 61.14; H, 7.70; N, 5.94%.

Example 12

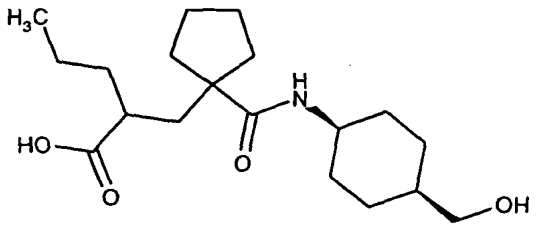
2-{[1-({[1-(Hydroxymethyl)cyclopentyl]amino}carbonyl)cyclopentyl]methyl}pentanoic acid



A solution of the *tert*-butyl ester from preparation 33 (38mg, 0.1mmol) in trifluoroacetic acid (2ml) and dichloromethane (2ml) was stirred at room temperature for 2 hours. The reaction was concentrated under reduced pressure and the residue azeotroped with toluene and then dichloromethane to give a colourless gum. This was suspended in a solution of potassium carbonate (50mg, 0.3mmol) in methanol, and the mixture stirred for 2 hours at room temperature. The methanol was removed under reduced pressure, the residual aqueous mixture diluted with water (20ml), and acidifed to pH 2 using 2N hydrochloric acid. This solution was extracted with ethyl acetate (2x20ml), and the combined organic solutions dried ($MgSO_4$), and evaporated under reduced pressure to give a clear oil, 32mg, 97%; ^1H NMR ($CDCl_3$, 400MHz) δ:: 0.88 (t, 3H), 1.20-1.40 (m, 3H), 1.41-1.90 (m, 17H), 2.01-2.20 (m, 2H), 2.40 (m, 1H), 3.71 (dd, 2H), 5.80 (bs, 1H); LRMS : m/z 326.1 (MH^+)

Example 13

Cis-2-{[1-({[4-(Hydroxymethyl)cyclohexyl]amino}carbonyl)cyclopentyl]methyl} pentanoic acid

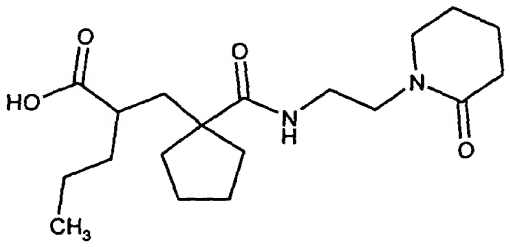


The title compound was obtained as a colourless gum in 68%, from the *tert*-butyl ester from preparation 43, following the procedure described in example 12, except the

product was additionally purified by column chromatography on silica gel using dichloromethane:methanol (95:5) as the eluant; ^{1}H NMR ($CDCl_3$, 400MHz) δ: 0.87 (t, 3H), 1.21-1.40 (m, 6H), 1.52-1.70 (m, 15H), 1.92-2.11 (m, 3H), 2.39 (m, 1H), 3.55 (d, 2H), 4.01 (m, 1H), 5.90 (m, 1H); LRMS : m/z 340.3 (MH^+).

Example 14

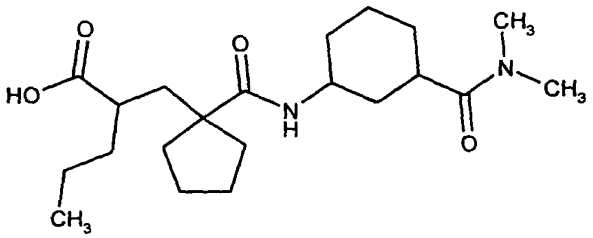
2-{[1-({[2-(2-Oxo-1-piperidinyl)ethyl]amino}carbonyl)cyclopentyl]methyl}pentanoic acid



Hydrogen chloride gas was bubbled through an ice-cold solution of the *tert*-butyl ester from preparation 47 (43mg, 0.105mmol) in dichloromethane (10ml), for 20 minutes. The solution was then stirred at room temperature for 3 hours. The mixture was concentrated under reduced pressure and the residue azeotroped with dichloromethane (3x), to give a glass-like solid. The crude product was purified by column chromatography on silica gel using an elution gradient of dichloromethane:methanol (95:5 to 90:10) to afford the title compound, 6mg; ^{1}H NMR ($CDCl_3$, 400MHz) δ: 0.81 (t, 3H), 1.20-1.36 (m, 4H), 1.41-1.69 (m, 7H), 1.79 (m, 4H), 1.90-2.10 (m, 3H), 2.30 (m, 1H), 2.38 (t, 2H), 3.30-3.60 (m, 6H), 7.00 (bs, 1H); LRMS : m/z 351 $(M-H)^-$.

Example 15

2-({1-[({3-[(Dimethylamino)carbonyl]cyclohexyl}amino)carbonyl]cyclopentyl}methyl) pentanoic acid

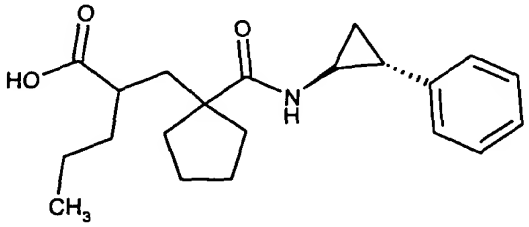


The title compound was obtained as a solid in 85% yield, from the *tert*-butyl ester from preparation 42, following a similar method to that described in example 14, except that dichloromethane:methanol:acetic acid (95:3:2) was used as the chromatographic

eluant; ^{1}H NMR ($CDCl_3$, 400MHz) d: 0.89 (t, 3H), 1.09-1.76 (m, 12H), 1.80-2.17 (m, 10H), 2.37 (m, 1H), 2.68 (m, 1H), 2.95 (s, 3H), 3.04 (s, 3H), 3.83 (m, 1H), 6.06 (m, 1H); LRMS : m/z 381 (MH^+); Anal. Found: C, 63.31; H, 9.17; N, 6.53. $C_{21}H_{36}N_2O_4;H_2O$ requires C, 63.29; H, 9.61; N, 7.03%.

Example 16

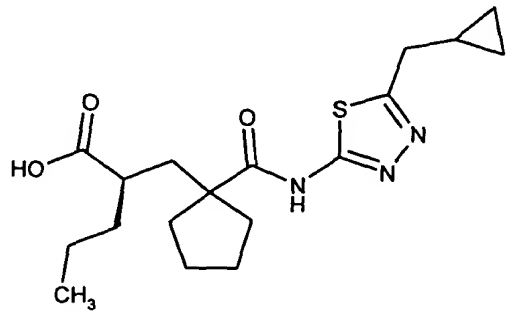
2-{[1-({[(1*R*,2*R*)-2-Phenylcyclopropyl]amino}carbonyl)cyclopentyl]methyl}pentanoic acid



The title compound was obtained quantitatively as an orange gum from the *tert*-butyl ester from preparation 46, following a similar procedure to that described in example 14; ^{1}H NMR ($CDCl_3$, 400MHz) δ: 0.90 (t, 3H), 1.12-2.14 (m, 17H), 2.38 (m, 1H), 2.87 (m, 1H), 6.10 (s, 1H), 7.13 (m, 3H), 7.25 (m, 2H); LRMS : m/z 344.3 (MH^+).

Example 17

(2*R*)-2-{[1-({[5-(Cyclopropylmethyl)-1,3,4-thiadiazol-2-yl]amino}carbonyl)cyclopentyl] methyl}pentanoic acid

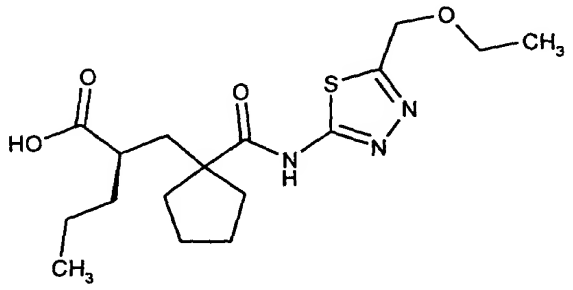


A solution of the *tert*-butyl ester from preparation 50 (63mg, 0.15mmol) in trifluoroacetic acid (2ml) and dichloromethane (2ml), was stirred at room temperature for 2 hours. The mixture was concentrated under reduced pressure and the residue purified by column chromatography on silica gel using dichloromethane:methanol (95:5) as eluant , to give the title compound as a white foam, 46mg, 83%; ^{1}H NMR (CD_3OD, 400MHz) δ: 0.38 (m, 2H), 0.62 (m, 2H), 0.82 (t, 3H), 1.12 (m, 1H), 1.26 (m,

2H), 1.38 (m, 1H), 1.52 (m, 1H), 1.78-1.78 (m, 6H), 1.90 (m, 1H), 2.23 (m, 4H), 2.92 (d, 2H); LRMS : m/z 366.0 (MH^+); $[\alpha]_D$ = -7.75° (c = 0.08, methanol).

Example 18

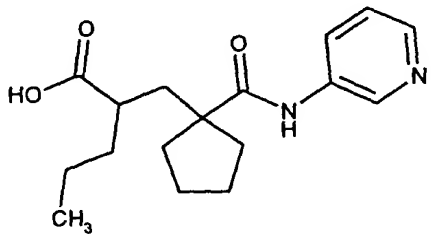
(2*R*)-2-{[1-({[5-(Ethoxymethyl)-1,3,4-thiadiazol-2-yl]amino}carbonyl)cyclopentyl]-methyl}pentanoic acid



The title compound was obtained as a white foam in 62% yield, from the *tert*-butyl ester from preparation 51, following a similar procedure to that described in example 17; ^1H NMR (CD_3OD, 400MHz) δ: 0.82 (t, 3H), 1.21-1.40 (m, 7H), 1.50 (m, 1H), 1.60-1.77 (m, 7H), 1.88 (m, 1H), 2.23 (m, 4H), 3.62 (q, 2H); $[\alpha]_D$ = -6.08° (c = 0.25, methanol).

Example 19

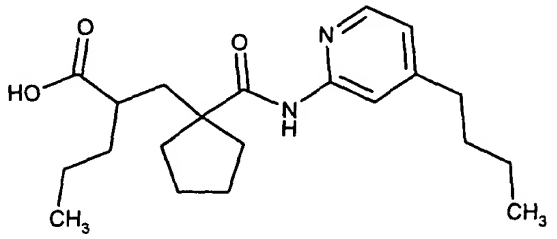
2-({1-[(3-Pyridinylamino)carbonyl]cyclopentyl}methyl)pentanoic acid



A mixture of the benzyl ester from preparation 52 (130mg, 0.33mmol) and 10% palladium on charcoal (20mg) in 95% aqueous ethanol (3ml) was hydrogenated at 15psi and room temperature for 2 hours. The reaction was filtered through Arbocel®, washing through with ethanol, and the filtrate evaporated under reduced pressure. The residual gum was purified by column chromatography on silica gel using dichloromethane:methanol (95:5) as eluant to afford the title compound, 103mg, 83%; ^1H NMR ($CDCl_3$, 400MHz) δ: 0.90 (t, 3H), 1.38 (m, 2H), 1.44 (m, 1H), 1.58-1.82 (m, 8H), 2.19 (m, 1H), 2.39 (m, 2H), 2.52 (m, 1H), 6.88 (m, 1H), 7.67 (m, 1H), 7.82 (d, 1H), 8.38 (d, 1H), 9.78 (s, 1H); LRMS : m/z 305 (MH^+).

Example 20

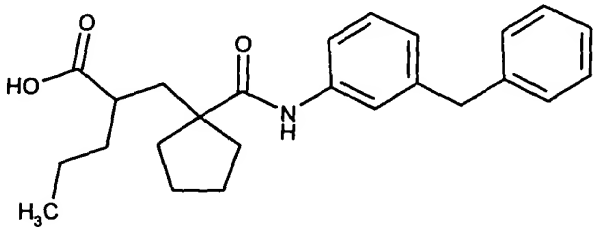
2-[(1-{[(4-Butyl-2-pyridinyl)amino]carbonyl}cyclopentyl)methyl]pentanoic acid



The title compound was obtained in 92% yield from the benzyl ester from preparation 55, following a similar procedure to that described in example 19; ^{1}H NMR ($CDCl_3$, 400MHz) δ: 0.90 (m, 6H), 1.28-1.50 (m, 5H), 1.58-1.81 (m, 10H), 2.20 (m, 1H), 2.40 (m, 2H), 2.58 (m, 3H), 6.70 (d, 1H), 7.68 (d, 1H), 8.22 (s, 1H), 9.90 (bs, 1H).

Example 21

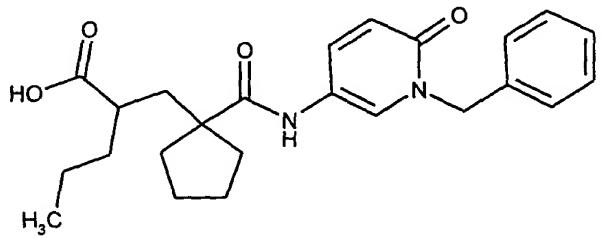
2-({1-[(3-Benzylanilino)carbonyl]cyclopentyl}methyl)pentanoic acid



A mixture of the benzyl ester from preparation 53 (1.3mg, 2.47mmol) and 5% palladium on charcoal (130mg) in water (10ml) and ethanol (40ml) was hydrogenated at 30 psi and room temperature for 2 hours. The reaction mixture was fiiltered through Arbocel®, the filtrate concentrated under reduced pressure, and the residue triturated with dichloromethane. The residual gum was triturated with ether, then hexane, and dried at 50°C, to give the title compound as a solid, 0.79g, 81%; ^{1}H NMR ($CDCl_3$, 300MHz) δ: 0.95 (t, 3H), 1.24-1.51 (m, 3H), 1.58-1.80 (m, 7H), 1.88 (dd, 1H), 2.15 (m, 2H), 2.24 (m, 1H), 2.48 (m, 1H), 4.00 (s, 2H), 6.98 (d, 1H), 7.24 (m, 6H), 7.40 (m, 3H); Anal. Found: C, 75.48; H, 7.76; N, 3.59. $C_{25}H_{31}NO_3$;0.25H_2O requires C, 75.44; H, 7.98; N, 3.51%.

Example 22

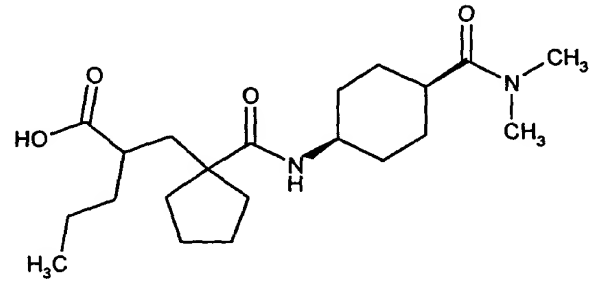
2-[(1-{[(1-Benzyl-6-oxo-1,6-dihydro-3-pyridinyl)amino]carbonyl}-cyclopentyl)methyl]-pentanoic acid.



The title compound was obtained as a white foam in 51% yield from the benzyl ester from preparation 56, following a similar procedure to that described in example 21, except, the product was purified by column chromatography on silica gel, using ethyl acetate as eluant; ^{1}H NMR ($CDCl_3$, 300MHz) δ: 0.96 (t, 3H), 1.28-1.80 (m, 12H), 2.01 (m, 1H), 2.30-2.52 (m, 2H), 5.02 (dd, 2H), 6.60 (d, 1H), 7.27 (m, 5H), 7.70 (s, 1H), 8.34 (s, 1H); Anal. Found: C, 69.52; H, 7.41; N, 6.51. $C_{24}H_{30}N_2O_4$;0.25H_2O requires C, 69.45; H, 7.41; N, 6.75.

Example 23

Cis-2-({1-[({4-[(Dimethylamino)carbonyl]cyclohexyl}amino)carbonyl]-cyclopentyl}methyl)pentanoic acid

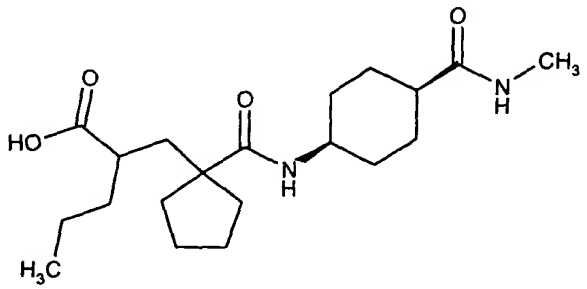


A mixture of the benzyl ester from preparation 58 (150mg, 0.33mmol) and 10% palladium on charcoal (20mg) in water (0.3ml) and ethanol (3.5ml) was hydrogenated at 15 psi and room temperature for 3 days. The reaction mixture was filtered through Arbocel®, and the filtrate concentrated under reduced pressure. The residual gum was purified by column chromatography on silica gel using dichloromethane:methanol (95:5) as eluant to afford the title compound, 85mg, 65%; ^{1}H NMR ($CDCl_3$, 400MHz) δ: 0.84 (t, 3H), 1.29-1.96 (m, 18H), 2.01-2.23 (m, 4H), 2.37 (m, 1H), 2.62 (m, 1H), 2.96 (s, 3H), 3.03 (s, 3H), 3.96 (m, 1H), 5.98 (m, 1H); LRMS : m/z 381.8 (MH^+); Anal.

Found: C, 63.81; H, 9.58; N, 6.99. $C_{21}H_{36}N_2O_4$;0.2CH_2Cl_2 requires C, 64.06; H, 9.23; N, 7.05%.

Example 24

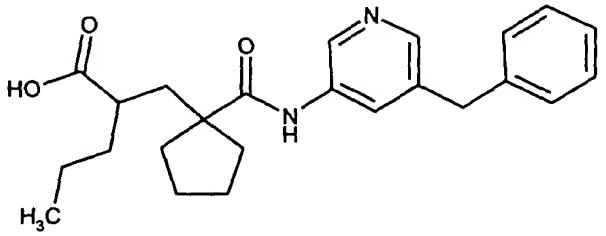
Cis-2-({1-[({4-[(Methylamino)carbonyl]cyclohexyl}amino)carbonyl]cyclopentyl}-methyl)pentanoic acid



The title compound was obtained as a white solid in 34% yield from the benzyl ester from preparation 59, following the procedure described in example 23; ^{1}H NMR ($CDCl_3$, 300MHz) δ: 0.90 (t, 3H), 1.26-2.02 (m, 20H), 2.19 (m, 3H), 2.39 (m, 1H), 2.82 (d, 3H), 4.00 (m, 1H), 5.69 (m, 1H), 6.00 (d, 1H); LRMS : m/z 365 ($M-H^-$).

Example 25

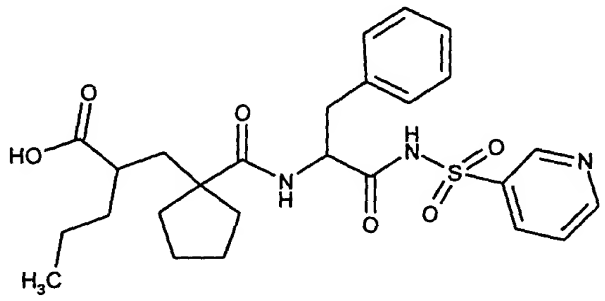
2-[(1-{[(5-Benzyl-3-pyridinyl)amino]carbonyl}cyclopentyl)methyl]-pentanoic acid.



A mixture of the benzyl ester from preparation 54 (850mg, 1.76mmol) and 5% palladium on charcoal (100mg) in 20% aqueous ethanol (30ml) was hydrogenated at 30 psi and room temperature for 2 hours. The mixture was filtered through Arbocel®, the filtrate evaporated under reduced pressure, and the residue azeotroped with dichloromethane to give the title compound as a foam, 0.63g; ^{1}H NMR ($CDCl_3$, 300MHz) δ: 0.92 (t, 3H), 1.30-1.83 (m, 11H), 2.07 (m, 1H), 2.42 (m, 3H), 3.82 (s, 2H), 7.15-7.38 (5H), 7.80 (s, 1H), 8.48 (s, 1H), 8.59 (s, 1H), 8.62 (s, 1H); Anal. Found: C, 72.29; H, 7.70; N, 6.90. $C_{24}H_{30}N_2O_3$;0.25H_2O requires C, 72.24; H, 7.70; N, 7.02%.

Example 26

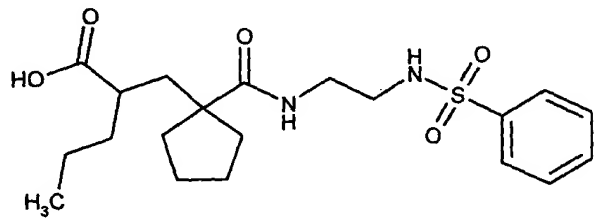
2-({1-[({1-Benzyl-2-oxo-2-[(3-pyridinylsulfonyl)amino]ethyl}amino)-carbonyl]cyclopentyl}methyl)pentanoic acid.



A mixture of the benzyl ester from preparation 57 (918mg, 1.52mmol) and 10% palladium on charcoal (90mg) in water (10ml) and ethanol (50ml) was hydrogenated at 50 psi and room temperature for 4 ½ hours. Tlc analysis showed starting material remaining, so additional catalyst (70mg) was added, and the mixture hydrogenated for a further 18 hours. Tlc analysis, again showed starting material remaining, so further catalyst (70mg) was added, and hydrogenation continued for an additional 6 hours. The reaction mixture was filtered through Arbocel®, the filtrate evaporated under reduced pressure and the residue azeotroped with dichloromethane. The crude product was purified by column chromatography on silica gel using an elution gradient of dichloromethane:acetic acid:ethanol (99:1:0 to 79.1:0.9:20) to afford the title compound as a white foam, 271mg, 35%; ^{1}H NMR (DMSOd_6, 300MHz) δ: 0.75 (m, 3H), 0.96-1.42 (m, 11H), 1.61-1.99 (m, 4H), 2.75-3.02 (m, 2H), 4.45 (m, 1H), 7.20 (m, 6H), 7.62 (m, 1H), 8.24 (m, 1H), 8.83 (s, 1H), 9.01 (s, 1H), 11.98 (bs, 1H), 12.70 (bs, 1H); IR (KBr disc) 1185, 1195 (m), 1455, 1515, 1640, 1704, 2870, 2930, 2960 (s).

Example 27

2-({1-[({2-[(Phenylsulfonyl)amino]ethyl}amino)carbonyl]cyclopentyl}methyl)pentanoic acid

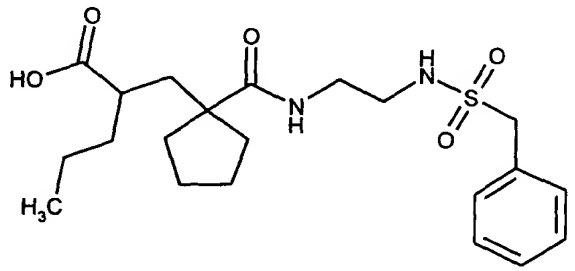


A mixture of the amine from preparation 61 (235mg, 0.72mmol), benzenesulphonyl chloride (127mg, 0.72mmol) and triethylamine (150µl, 1.08mmol) in dichloromethane

(6ml) was stirred at room temperature for 2 days. The mixture was concentrated under reduced pressure and the residue purified by column chromatography on silica gel using ethyl acetate:pentane (30:70) as eluant to give a clear oil. This was then dissolved in trifluoroacetic acid (3ml) and dichloromethane (3ml) and the solution stirred at room temperature for 6 hours. The mixture was concentrated under reduced pressure and the residue azeotroped twice with toluene. The crude product was purified by column chromatography on silica gel using ethyl acetate:pentane (30:70) to afford the title compound as a clear oil, 204mg, 69%; ^{1}H NMR ($CDCl_3$, 400MHz) δ: 0.84 (t, 3H), 1.22-1.43 (m, 4H), 1.43-2.18 (m, 10H), 2.36 (m, 1H), 3.11 (m, 2H), 3.20-3.31 (m, 1H), 3.42-3.53 (m, 1H), 6.13-6.24 (m, 1H), 7.42-7.59 (m, 3H), 7.84 (m, 2H); LRMS : m/z 411.8 (MH^+); Anal. Found: C, 57.26; H, 7.40; N, 6.61. $C_{20}H_{30}N_2O_5S$ requires C, 57.18; H, 7.22; N, 6.62%.

Example 28

2-({1-[({2-[(Benzylsulfonyl)amino]ethyl}amino)carbonyl]cyclopentyl}methyl)pentanoic acid

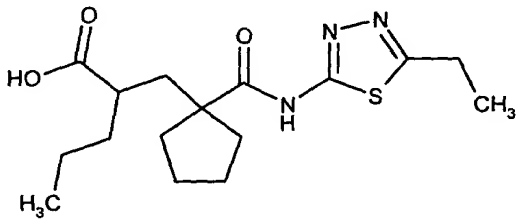


The title compound was obtained as a clear oil in 97% yield, from the amine from preparation 61, following the procedure described in example 27, ^{1}H NMR ($CDCl_3$, 300MHz) δ: 0.87 (t, 3H), 1.19-1.72 (m, 11H), 1.80-1.96 (m, 1H), 2.00-2.16 (m, 2H), 2.27-2.38 (m, 1H), 2.92-3.21 (m, 3H), 3.23-3.39 (m, 1H), 4.25 (s, 2H), 5.80-6.06 (m, 1H), 6.38 (m, 1H), 7.29-7.43 (m, 5H); LRMS : m/z 425.8 (MH^+).

Example 29
(-)-2-[(1-{[(5-Ethyl-1,3,4-thiadiazol-2-yl)amino]carbonyl}cyclopentyl)methyl]pentanoic acid

and

Example 30
(+)-2-[(1-{[(5-Ethyl-1,3,4-thiadiazol-2-yl)amino]carbonyl}cyclopentyl)methyl]pentanoic acid



The acid from Example 4 (824mg) was further purified by HPLC using an AD column and using hexane:*iso*-propanol:trifluoroacetic acid (85:15:0.2) as eluant to give the title compound of example 29 as a white foam, 400mg, 99.5% ee, ^{1}H NMR ($CDCl_3$, 400MHz) δ: 0.90 (t, 3H), 1.36 (m, 6H), 1.50-1.80 (m, 9H), 2.19 (m, 1H), 2.30 (m, 1H), 2.44 (m, 1H), 2.60 (m, 1H), 2.98 (q, 2H), 12.10-12.30 (bs, 1H), LRMS : m/z 338 (MH^-), $[\alpha]_D$ = -9.0° (c = 0.1, methanol), and the title compound of example 30 as a white foam, 386mg, 99% ee, ^{1}H NMR ($CDCl_3$, 400MHz) δ: 0.90 (t, 3H), 1.38 (m, 6H), 1.50-1.79 (m, 9H), 2.19 (m, 1H), 2.30 (m, 1H), 2.44 (m, 1H), 2.60 (m, 1H), 2.98 (q, 2H), 12.10-12.27 (bs, 1H); LRMS: m/z 338 (MH^-); and $[\alpha]_D$ = +3.8° (c = 0.1, methanol)

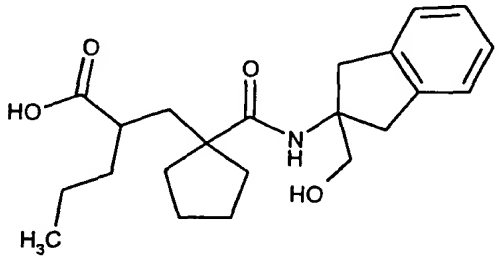
Example 31

(+)-2-{[1-({[2-(Hydroxymethyl)-2,3-dihydro-1*H*-inden-2-yl]amino}carbonyl)cyclopentyl]-methyl}pentanoic acid

and

Example 32

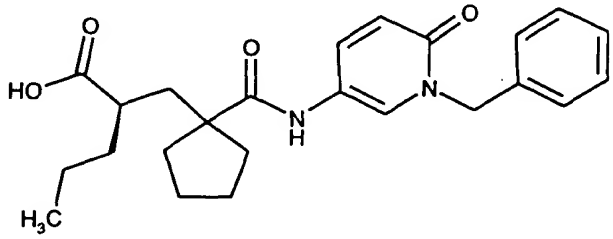
(-)-2-{[1-({[2-(Hydroxymethyl)-2,3-dihydro-1H-inden-2-yl]amino}carbonyl)cyclopentyl]-methyl}pentanoic acid



2-{[1-({[2-(Hydroxymethyl)-2,3-dihydro-1*H*-inden-2-yl]amino}carbonyl)cyclopentyl]-methyl}pentanoic acid (WO 9110644) was further purified by HPLC using an AD column and hexane:isopropanol:trifluoroacetic acid (90:10:0.1) as eluant, to give the title compound of example 31, 99% ee, $[\alpha]_D$ = +10.4° (c = 0.067, ethanol) and the title compound of example 32, 99% ee, $[\alpha]_D$ = -10.9° (c = 0.046, ethanol).

Example 33

(2*R*)-2-[(1-{[(1-Benzyl-6-oxo-1,6-dihydro-3-pyridinyl)amino]carbonyl}-cyclopentyl)methyl]-pentanoic acid.

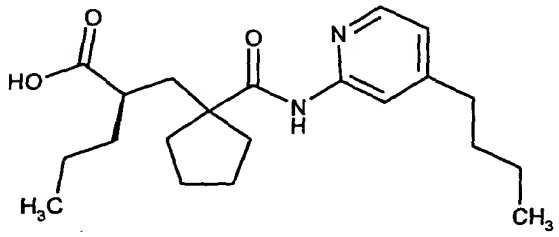


1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (191mg, 1.0mmol), 1-hydroxybenzotriazole hydrate (135mg, 01.0mmol), N-methylmorpholine (165µl, 1.5mmol) and finally the amine from preparation 28 (150mg, 0.69mmol) were added to a solution of the acid from preparation 2 (284mg, 1.0mmol) in N,N-dimethylformamide (8ml), and the reaction stirred at 90°C for 18 hours. The cooled solution was diluted with ethyl acetate (90ml), washed with water (4x50ml), and brine (50ml), then dried ($MgSO_4$) and evaporated under reduced pressure. The crude product was purified by chromatography on silica gel, using ethyl acetate:pentane (30:70) to give a yellow oil,

191mg. This intermediate was dissolved in dichloromethane (3ml) and trifluoroacetic acid (3ml) and the solution stirred at room temperature for 5 hours. The mixture was concentrated under reduced pressure and the residue purified by column chromatography on silica gel using dichloromethane:methanol (95:5) as eluant to give the title compound as a foam, 77mg, ^{1}H NMR ($CDCl_3$, 300MHz) δ: 0.86 (t, 3H), 1.20-1.76 (m, 12H), 1.93-2.02 (m, 1H), 2.20-2.46 (m, 3H), 4.95 (d, 1H), 5.04 (d, 1H), 6.61 (d, 1H), 7.21 (m, 1H), 7.50 (s, 1H), 8.23 (s, 1H); LRMS : m/z 411.6 (MH)+; $[\alpha]_D$ = -3.8° (c = 0.052, ethanol).

Example 34

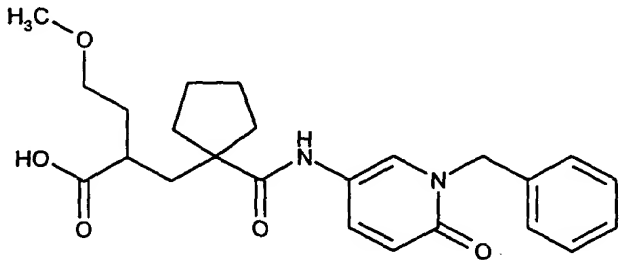
(2*R*)-2-[(1-{[(4-Butyl-2-pyridinyl)amino]carbonyl}cyclopentyl)methyl]pentanoic acid



The title compound was obtained in 43% yield from the acid from preparation 2 and the amine from preparation 30, following a similar procedure to that described in example 33, ^{1}H NMR ($CDCl_3$, 400MHz) δ: 0.80-1.00 (m, 6H), 1.22-1.84 (m, 18H), 2.03-2.56 (m, 3H), 2.77 (m, 1H), 7.14 (d, 1H), 8.08 (d, 1H), 8.23 (s, 1H), 11.71 (brs, 1H). LRMS : m/z 361.7 $(MH)^+$, $[\alpha]_D$ = -1.4° (c = 0.14, ethanol).

Example 35

2-[(1-{[(1-Benzyl-6-oxo-1,6-dihydro-3-pyridinyl)amino]carbonyl}cyclopentyl)methyl]-4-methoxybutanoic acid

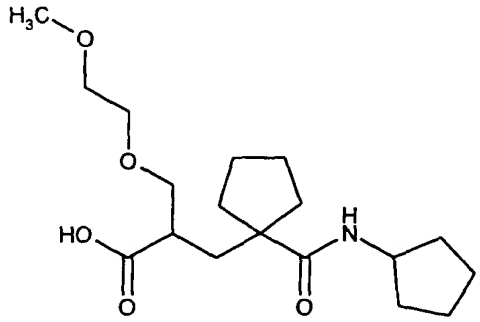


A mixture of the benzyl ester from preparation 62 (850mg, 1.64mmol), and 5% palladium on charcoal (250mg) in 40% aqueous ethanol (21ml), was hydrogenated at 30 psi and room temperature for 30 minutes. The reaction mixture was filtered through Hyflo®, and the filtrate evaporated under reduced pressure. The residual foam was

purified by column chromatography on silica gel using dichloromethane:methanol (97:3) as eluant to give the title compound as a white foam, 550mg, 79%; ^{1}H NMR (DMSO-d_6, 300MHz) δ: 1.24-2.17 (m, 12H), 2.18-2.31 (m, 1H), 3.07 (s, 3H), 3.21 (t, 2H), 5.08 (s, 2H), 6.63 (d, 1H), 7.23-7.41 (m, 5H), 7.72 (d, 1H), 8.24 (s, 1H). Anal. Found: C, 67.46; H, 7.18; N, 6.24. $C_{24}H_{30}N_2O_5$ requires C, 67.58; H, 7.09; N, 6.57%.

Example 36

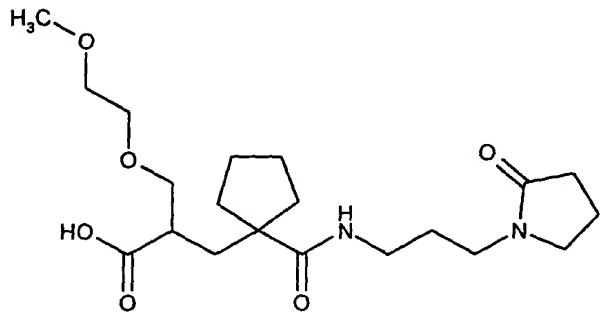
3-{1-[(Cyclopentylamino)carbonyl]cyclopentyl}-2-[(2-methoxyethoxy)methyl]propanoic acid



A solution of the *tert*-butyl ester from preparation 64 (320mg, 0.80mmol) in trifluoroacetic acid (2ml) and dichloromethane (2ml) was stirred at room temperature for 8 hours. The mixture was concentrated under reduced pressure and the residue azeotroped twice with toluene. The crude product was purified by column chromatography on silica gel using dichloromethane:methanol (95:5) to give the title compound as a clear oil, 171mg, 62%; ^{1}H NMR ($CDCl_3$, 400MHz) δ: 1.29-1.40 (m, 2H), 1.42-1.69 (m, 10H), 1.75 (dd, 1H), 1.87-2.03 (m, 5H), 2.64 (m, 1H), 3.34 (s, 3H), 3.43-3.52 (m, 3H), 3.57 (m, 2H), 3.61 (m, 1H), 4.08-4.20 (m, 1H), 5.89 (d, 1H); LRMS : m/z 340 (MH^-).

Example 37

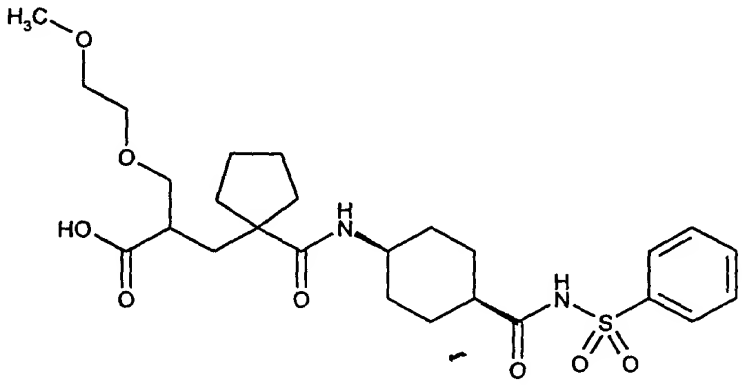
3-(2-Methoxyethoxy)-2-{[1-({[3-(2-oxo-1-pyrrolidinyl)propyl]amino}carbonyl)-cyclopentyl]methyl}propanoic acid



The title compound was obtained as a clear oil in 57% yield from the *tert*-butyl ester of preparation 65, following the procedure described in example 36, ^{1}H NMR ($CDCl_3$, 300MHz) δ: 1.56-1.78 (m, 8H), 1.94-2.17 (m, 6H), 2.44 (m, 2H), 2.68-2.76 (m, 1H), 3.10-3.21 (m, 1H), 3.22-3.31 (m, 1H), 3.37 (s, 3H), 3.40 (m, 2H), 3.44-3.56 (m, 5H), 3.60 (m, 2H), 3.68 (m, 1H), 6.91-7.01 (m, 1H); LRMS : m/z 398.7 (M^+)

Example 38

Cis-3-(2-Methoxyethoxy)-2-[(1-{[(4-{[(phenylsulfonyl)amino]carbonyl}cyclohexyl)-amino]carbonyl}cyclopentyl)methyl]propanoic acid

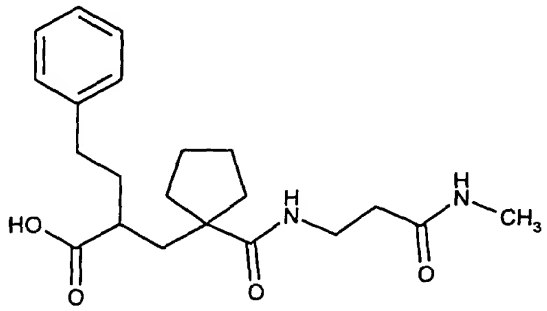


A solution of the *tert*-butyl ester from preparation 66 (446mg, 0.75mmol) in dichloromethane (5ml) and trifluoroacetic acid (5ml) was stirred at room temperature for 18 hours. The reaction mixture was concentrated under reduced pressure, and the residue azeotroped with dichloromethane, then toluene, and finally ether, to afford the title compound as a white foam, 385mg, 95%; ^{1}H NMR ($CDCl_3$, 400MHz) δ: 1.48-2.17 (m, 18H), 2.40 (s, 1H), 2.66 (s, 1H), 3.37 (s, 3H), 3.50-3.70 (m, 6H), 3.94 (s, 1H), 6.10 (d, 1H), 6.59 (s, 1H), 7.55 (t, 2H), 7.61 (m, 1H), 8.02 (d, 2H), 9.11 (s, 1H); Anal. Found: C, 54.88; H, 6.90; N, 5.04. $C_{26}H_{38}N_2O_8S;1.7H_2O$ requires C, 57.97; H, 7.11; N,

5.20%.

Example 39

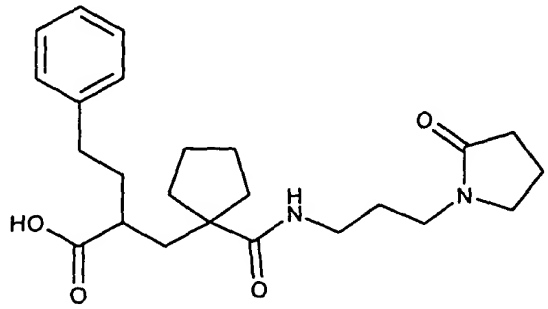
2-{[1-({[3-(Methylamino)-3-oxopropyl]amino}carbonyl)cyclopentyl]methyl}-4-phenylbutanoic acid



A mixture of the benzyl ester from preparation 68 (160mg, 0.34mmol) and 10% palladium on charcoal (100mg) in ethanol (30ml) was hydrogenated at room temperature and 60 psi for 18 hours. The mixture was filtered through Arbocel® and the filtrate concentrated under reduced pressure, and azeotroped with dichloromethane. The crude product was purified by column chromatography on silica gel using an elution gradient of dichloromethane:methanol:acetic acid (95:5:0 to 95:5:0.5) to afford the title compound as a white foam, 100mg, 79%; ^{1}H NMR ($CDCl_3$, 400MHz) δ: 1.40-1.70 (m, 8H), 1.95 (m, 3H), 2.10 (m, 1H), 2.35 (d, 3H), 2.59 (m, 2H), 2.75 (t, 3H), 3.42 (m, 2H), 6.25 (bs, 1H), 6.70 (bs, 1H), 7.13-7.25 (m, 5H); and LRMS: m/z 375.0 (MH^+).

Example 40

2-{[1-({[3-(2-Oxo-1-pyrrolidinyl)propyl]amino}carbonylcyclopentyl]-methyl}-4-phenylbutanoic acid.

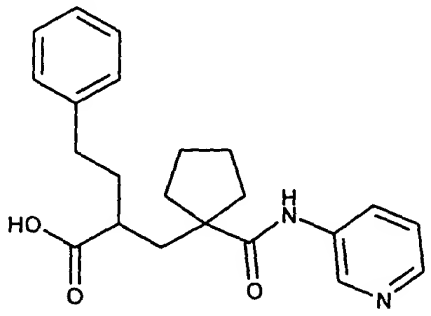


A mixture of the benzyl ester from preparation 67 (780mg, 1.55mmol) and 10% palladium on charcoal (100mg) in ethanol:water (90:10 by volume), (30ml) was

hydrogenated at room temperature under 60psi H_2 pressure for 1.5 hours. The catalyst was filtered off, and the filtrate evaporated under reduced pressure to provide the title compound as a white foam, 473mg, 74%; ^{1}H NMR ($CDCl_3$, 300MHz) d : 1.26-1.77 (m, 10H), 1.78-2.46 (m, 11H), 2.49-2.70 (m, 2H), 2.95-3.36 (m, 4H), 6.92-7.38 (m, 5H); Anal. Found: C, 64.05; H, 7.73; N, 6.22. $C_{24}H_{34}N_2O_4$;0.75H_2O requires C, 65.88; H, 7.83; N, 6.40%.

Example 41

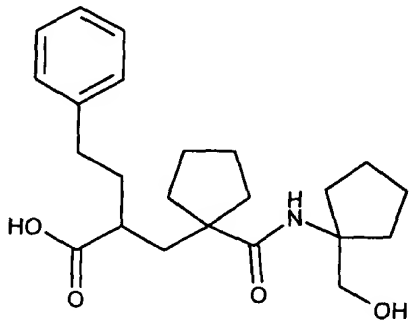
4-Phenyl-2-({1-[(3-pyridinylamino)carbonyl]cyclopentyl}methyl)butanoic acid



A mixture of the benzyl ester from preparation 71 (700mg, 1.53mmol) and 5% palladium on charcoal (70mg) in ethanol:water (90:10 by volume, 50ml) was hydrogenated at room temperature under 30 psi H_2 pressure for 5 hours. The catalyst was filtered through Arbocel®, washing well with ethanol, and the filtrate evaporated under reduced pressure. The crude product was purified by column chromatography on silica gel using dichloromethane:methanol (95:5) as the eluant to provide the title compound as a white foam, 510mg, 91%; mp 80-85°C (collapses to a gum); ^{1}H NMR ($CDCl_3$, 300MHz) δ: 1.40-2.78 (m, 15H), 6.93-7.39 (m, 5H), 7.93 (m, 1H), 8.59 (d, 1H), 9.17 (d, 1H), 9.41 (s, 1H); Anal. Found: C, 70.83; H, 7.10; N, 7.64. $C_{22}H_{26}N_2O_3$;0.3H_2O requires C, 70.94; H, 7.22; N, 7.52%.

Example 42

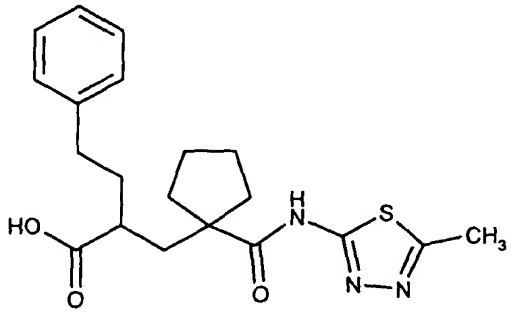
2-{[1-({[1-(Hydroxymethyl)cyclopentyl]amino}carbonyl)cyclopentyl]methyl}-4-phenylbutanoic acid



A mixture of the benzyl ester from preparation 69 (118mg, 0.25mmol) and 10% palladium on charcoal (100mg) in ethanol (20ml) was hydrogenated at room temperature and 60 psi for 18 hours. The mixture was filtered through Arbocel®, the filtrate concentrated under reduced pressure, and azeotroped with dichloromethane to give the title compound as a colourless gum, 95mg, 98%; ^{1}H NMR ($CDCl_3$, 300MHz) δ: 1.41-1.80 (m, 17H), 1.90 (m, 1H), 1.92-2.20 (m, 3H), 2.40 (m, 1H), 2.60 (m, 2H), 3.60 (d, 1H), 3.71 (d, 1H), 5.80 (bs, 1H), 7.15-7.30 (m, 5H); LRMS : m/z 388.1 (MH^+)

Example 43

2-[(1-{[(5-Methyl-1,3,4-thiadiazol-2-yl)amino]carbonyl}cyclopentyl)methyl]-4-phenylbutanoic acid



A mixture of the benzyl ester from preparation 70 (187mg, 0.39mmol) and 10% palladium on charcoal (80mg) in ethanol (20ml) was hydrogenated at 60 psi for 18 hours. Tlc analysis showed starting material remaining, so additional 10% palladium on charcoal (100mg) was added, and the reaction continued for a further 5 hours. Tlc analysis again showed starting material remaining, so additional catalyst (100mg) was added, and hydrogenation continued for 18 hours. The mixture was filtered through Arbocel®, and the filtrate concentrated under reduced pressure, and azeotroped with

dichloromethane. The crude product was purified by chromatography on silica gel using a Biotage® column, and dichloromethane:methanol (95:5) as eluant to afford the title compound as a clear oil, 80mg, 53%; ^{1}H NMR ($CDCl_3$, 300MHz) δ: 1.51-1.89 (m, 9H), 2.03 (m, 1H), 2.20 (m, 1H), 2.40 (m, 2H), 2.60 (m, 5H), 7.15-7.30 (m, 5H); LRMS : m/z 387.8 (MH^+).

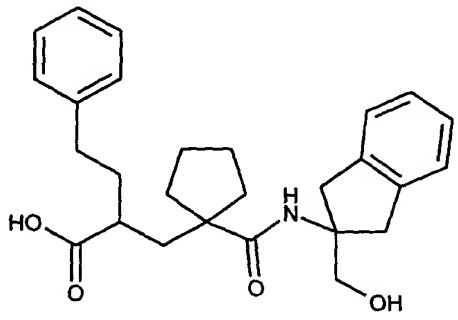
Example 44

(+)-2-{[1-({[2-(Hydroxymethyl)-2,3-dihydro-1*H*-inden-2-yl]amino}carbonyl)cyclopentyl]-methyl}-4-phenylbutanoic acid

and

Example 45

(-)-2-{[1-({[2-(Hydroxymethyl)-2,3-dihydro-1*H*-inden-2-yl]amino}carbonyl)cyclopentyl]methyl}-4-phenylbutanoic acid



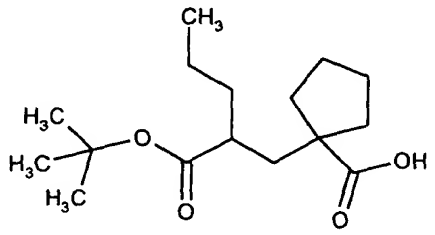
2-{[1-({[2-(Hydroxymethyl)-2,3-dihydro-1*H*-inden-2-yl]amino}carbonyl)cyclopentyl]-methyl}-4-phenylbutanoic acid (WO 9110644) may be purified by standard HPLC procedures using an AD column and hexane:isopropanol:trifluoroacetic acid (70:30:0.2) as eluant, to give the title compound of example 44, 99.5% ee; $[\alpha]_D$ = +9.1° (c = 1.76 in ethanol); and the title compound of example 45, 99.5% ee; $[\alpha]_D$ = -10.5° (c = 2.2 in ethanol).

The following Preparations describe the preparation of certain intermediates used in the preceding Examples.

Preparations

Preparation 1

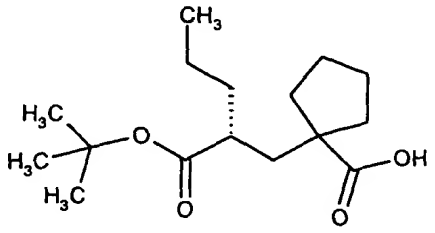
1-[2-(*tert*-Butoxycarbonyl)-4-pentyl]-cyclopentane carboxylic acid



A mixture of 1-[2-(*tert*-butoxycarbonyl)-4-pentenyl]-cyclopentane carboxylic acid (EP 274234) (23g, 81.5mmol) and 10% palladium on charcoal (2g) in dry ethanol (200ml) was hydrogenated at 30psi and room temperature for 18 hours. The reaction mixture was filtered through Arbocel®, and the filtrate evaporated under reduced pressure to give a yellow oil. The crude product was purified by column chromatography on silica gel, using ethyl acetate:pentane (40:60) as the eluant, to provide the desired product as a clear oil, 21g, 91%; ^{1}H NMR ($CDCl_3$, 0.86 (t, 3H), 1.22-1.58 (m, 15H), 1.64 (m, 4H), 1.78 (dd, 1H), 2.00-2.18 (m, 3H), 2.24 (m, 1H); LRMS : m/z 283 $(M-H)^-$

Preparation 2

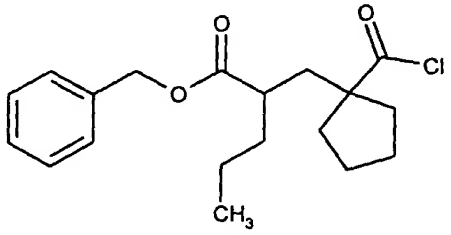
1-[(2*R*)-2-(*tert*-Butoxycarbonyl)-4-pentyl]-cyclopentane carboxylic acid



A mixture of (R)-1-[2-(*tert*-butoxycarbonyl)-4-pentenyl]-cyclopentane carboxylic acid (WO 9113054) (10g, 35.4mmol) and 10% palladium on charcoal (600mg) in dry ethanol (25ml) was hydrogenated at 1 atm. and room temperature for 18 hours. The reaction mixture was filtered through Arbocel®, and the filtrate evaporated under reduced pressure to give the title compound as a yellow oil, 9.6g, 95%; ^{1}H NMR ($CDCl_3$, 0.86 (t, 3H), 1.22-1.58 (m, 15H), 1.64 (m, 4H), 1.78 (dd, 1H), 2.00-2.18 (m, 3H), 2.24 (m, 1H); $[\alpha]_D$ = -3.3° (c = 0.09, ethanol).

Preparation 3

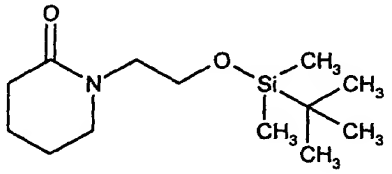
Benzyl 2-{[1-(chlorocarbonyl)cyclopentyl]methyl}pentanoate



Oxalyl chloride (1.15ml, 13.2mmol) was added to an ice-cooled solution of 1-{2-[(benzyloxy)carbonyl]pentyl}cyclopentanecarboxylic acid (EP 274234) (2.0g, 6.3mmol) in dry dichloromethane (20ml), and the solution stirred at room temperature for 2 hours. The reaction mixture was concentrated under reduced pressure and the residue azeotroped with dichloromethane (3x), to give the title compound as a golden oil, 2.1g; ^{1}H NMR ($CDCl_3$, 300MHz) δ: 0.88 (t, 3H), 1.28 (m, 2H), 1.43 (m, 2H), 1.63 (m, 6H), 2.00 (m, 1H), 2.08-2.35 (m, 3H), 2.44 (m, 1H), 5.15 (s, 2H), 7.28 (m, 5H).

Preparation 4

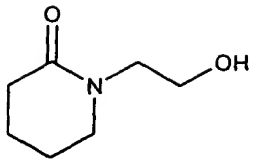
1-(2-{[*tert*-Butyl(dimethyl)silyl]oxy}ethyl)-2-piperidinone



Sodium hydride (807mg, 60% dispersion in mineral oil, 20.18mmol) was added portionwise to a solution of d-valerolactam (2.0g, 20.2mmol) in tetrahydrofuran (100ml) under nitrogen. (2-Bromoethoxy)(*tert*-butyl)dimethylsilane (4.33ml, 20.2mmol) was added portionwise, and the reaction heated at 70°C for 18 hours. Water (50ml) was added to the cooled reaction, the mixture concentrated *in vacuo*, to remove the tetrahydrofuran, and extracted with ethyl acetate (200ml). The organic solution was dried ($MgSO_4$), and evaporated under reduced pressure to give a yellow oil. The crude product was purified by column chromatography on silica gel using an elution gradient of dichloromethane:methanol (98:2 to 97:3) to give the title compound, 3.25g; ^{1}H NMR ($CDCl_3$, 400MHz) δ: 0.00 (s, 6H), 0.83 (s, 9H), 1.75 (m, 4H), 2.35 (m, 2H), 3.39 (m, 4H), 3.75 (t, 2H); LRMS : m/z 257.9 (M^+)

Preparation 5

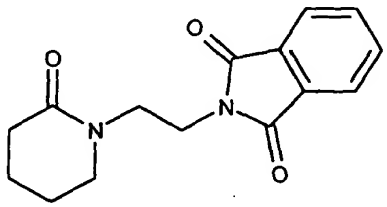
1-(2-Hydroxyethyl)-2-piperidinone



Tetra-n-butylammonium fluoride (14ml, 1M solution in tetrahydrofuran, 14mmol) was added to a solution of the lactam from preparation 4 (3.3g, 12.8mmol) in tetrahydrofuran (50ml), and the reaction stirred at room temperature for 2 hours. The mixture was concentrated under reduced pressure, the residue azeotroped with dichloromethane, and purified by column chromatography on silica gel using an elution gradient of dichloromethane:methanol (97:3 to 95:5) to give the title compound as an oil; ^{1}H NMR ($CDCl_3$, 400MHz) δ: 1.80 (m, 4H), 2.40 (t, 2H), 3.38 (t, 2H), 3.42 (t, 1H), 3.56 (t, 2H), 3.80 (t, 2H).

Preparation 6

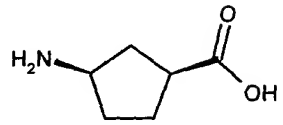
2-[2-(2-Oxo-1-piperidinyl)ethyl]-1*H*-isoindole-1,3(2*H*)-dione



Pthalimide (952mg, 6.47mmol) was added to a solution of the alcohol from preparation 5 (842mg, 5.88mmol) in tetrahydrofuran (30ml), and the mixture sonicated until a solution was obtained. Polymer supported triphenyl phosphine (2.5g, 7.5mmol) and diethyl azodicarboxylate (1.15ml, 7.31mmol) were added, and the reaction stirred at room temperature for 18 hours. The mixture was filtered through Arbocel®, the filtrate concentrated under reduced pressure and the residue azeotroped with dichloromethane. The crude product was purified by column chromatography on silica gel using an elution gradient of ethyl acetate:pentane (70:30 to 100:0), to give the title compound as a white foam, 1.6g (containing some impurities); ^{1}H NMR ($CDCl_3$, 400MHz) δ: 1.60-1.80 (m, 4H), 2.17 (m, 2H), 3.30 (m, 2H), 3.60 (m, 2H), 3.83 (m, 2H), 7.62 (m, 2H), 7.79 (m, 2H); LRMS : m/z 273.2 (MH^+).

Preparation 7

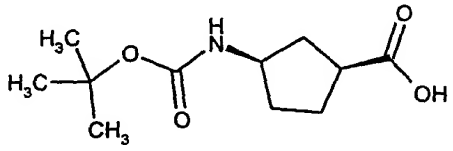
(1*S*,3*R*)-3-Aminocyclopentanecarboxylic acid



Platinum oxide (1g) was added to a solution of (1*R*,4*S*)-4-amino-cyclopent-2-ene carboxylic acid (5.3g, 41.7mmol) in water (70ml), and the mixture was hydrogenated at 45 psi and room temperature for 18 hours. The mixture was filtered through Arbocel®, the filtrate evaporated under reduced pressure, and the residue azeotroped with toluene, to afford the title compound as an off-white solid; ^{1}H NMR (D_2O, 400MHz) δ: 1.70-1.92 (m, 3H), 2.00 (m, 2H), 2.18 (m, 1H), 2.77 (m, 1H), 3.68 (m, 1H); LRMS : m/z 129.8 (MH^+).

Preparation 8

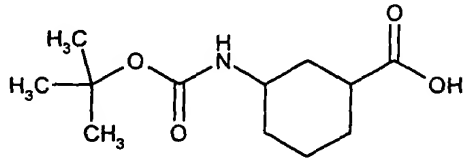
(1*S*,3*R*)-3-[(*tert*-Butoxycarbonyl)amino]cyclopentanecarboxylic acid



Di-*tert*-butyl dicarbonate (10g, 45.8mmol) was added to an ice-cooled solution of the amino acid from preparation 7 (5.4g, 41.8mmol) in dioxan (42.5ml) and sodium hydroxide solution (42.5ml, 1N, 42.5mmol), and the reaction stirred at room temperature for 18 hours. The reaction mixture was concentrated under reduced pressure to remove the dioxan, then acidifed to pH 2 using 2N hydrochloric acid. The aqueous solution was extracted with ethyl acetate (5x100ml), the combined organic extracts dried ($MgSO_4$) and evaporated under reduced pressure to give a white solid. This was triturated with hexane, to give the desired compound as a crystalline solid, 8.0g, 83%; ^{1}H NMR ($CDCl_3$, 400MHz) δ: 1.41 (s, 9H), 1.58-2.06 (m, 5H), 2.21 (m, 1H), 2.84 (m, 1H), 4.01 (m, 1H), 4.84 (m, 1H); LRMS: m/z 228 $(M-H)^-$.

Preparation 9

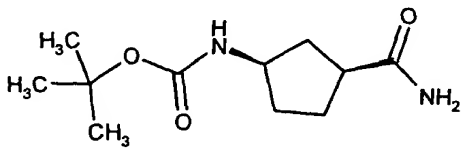
3-[(*tert*-Butoxycarbonyl)amino]cyclohexanecarboxylic acid



The title compound was obtained as a white solid in 81% yield, from 3-aminocyclohexanecarboxylic acid, following the procedure described in preparation 8; ^{1}H NMR ($CDCl_3$, 400MHz) δ: 1.04 (m, 1H), 1.19-1.50 (m, 13H), 1.83 (m, 1H), 1.97 (m, 2H), 2.24 (m, 1H), 2.40 (m, 1H), 3.44 (bs, 1H), 4.42 (bs, 1H).

Preparation 10

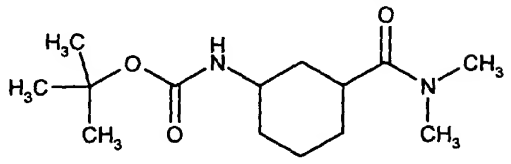
tert-Butyl (1*R*,3*S*)-3-(aminocarbonyl)cyclopentylcarbamate



Benzotriazol-1-yloxytris(pyrrolidino)phosphonium hexafluorophosphate (3.4g, 6.54mmol), 1-hydroxybenzotriazole hydrate (883mg, 6.54mmol), ammonium chloride (467mg, 8.72mmol) and N-ethyldiisopropylamine (3.04ml, 17.5mmol) were added sequentially to a solution of the acid from preparation 8 (1.0g, 4.37mmol) in N,N-dimethylformamide (16ml), and the reaction stirred at room temperature for 2 hours. The mixture was diluted with ethyl acetate (100ml), washed with water (3x), and brine, then dried ($MgSO_4$) and evaporated under reduced pressure. The residual gum was purified by chromatography on silica gel using a Biotage® column, and an elution gradient of dichloromethane:methanol (98:2 to 95:5). The product was triturated with ether to afford the title compound as a white solid, 438mg, 44%; ^{1}H NMR ($DMSOd_6$, 400MHz) δ: 1.34 (s, 9H), 1.40 (m, 2H), 1.64 (m, 3H), 1.90 (m, 1H), 2.55 (m, 1H), 3.70 (m, 1H), 6.70 (bs, 1H), 6.80 (d, 1H), 7.22 (bs, 1H).

Preparation 11

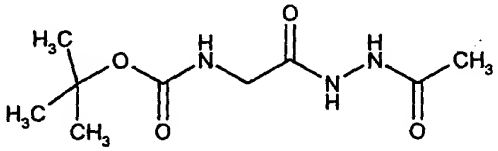
tert-Butyl 3-[(dimethylamino)carbonyl]cyclohexylcarbamate



1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (1.19g, 6.19mmol), 1-hydroxybenzotriazole hydrate (840mg, 6.19mmol), N-methylmorpholine (1.1ml, 10.1mmol) and finally 33% ethanolic dimethylamine (1.5ml) were added to a solution of the acid from preparation 9 (1.37g, 5.6mmol) in N,N-dimethylformamide (30ml), and the reaction stirred at room temperature for 18 hours. The mixture was concentrated under reduced pressure, the residue diluted with ethyl acetate and washed with water (2x). The mixture was dried ($MgSO_4$) and evaporated under reduced pressure. The crude product was purified by column chromatography on silica gel using an elution gradient of methanol:dichloromethane (5:95 to 10:90), to give the title compound, 998mg, 66%; ^{1}H NMR ($CDCl_3$, 300MHz) δ: 1.12 (m, 1H), 1.40 (m, 11H), 1.70 (m, 2H), 1.85 (m, 1H), 2.00 (m, 2H), 2.62 (m, 1H), 2.96 (s, 3H), 3.05 (s, 3H), 3.50 (m, 1H), 4.50 (m, 1H).

Preparation 12

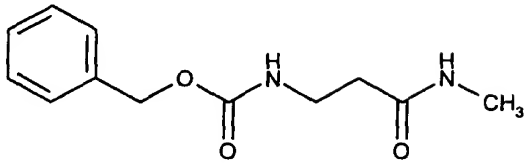
tert-Butyl 2-(2-acetylhydrazino)-2-oxoethylcarbamate



2-Ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline (7.06g, 28.5mmol) was added to a solution of N-*tert*-butoxycarbonylglycine (5.0g, 28.6mmol) in dichloromethane (75ml), and the solution stirred for 15 minutes. Acetic hydrazide (2.6g, 35.1mmol) was added, and the reaction stirred at room temperature for 18 hours. The resulting precipitate was filtered off, and dried *in vacuo*, to afford a white crystalline solid, 2.42g. The filtrate was concentrated under reduced pressure, diluted with ether, and the resulting precipitate filtered and dried *in vacuo*, to afford additional product as a white solid, 4.4g, 67% in total; ^{1}H NMR ($CDCl_3$, 400MHz) δ: 1.41 (s, 9H), 2.02 (s, 3H), 3.87 (d, 2H), 5.22 (bs, 1H), 8.27 (bs, 1H), 8.84 (bs, 1H); LRMS : m/z 249.2 (MNH_4^+); Anal. Found: C, 46.41; H, 7.36; N, 17.98, $C_9H_{17}N_3O_4$ requires C, 46.66; H, 7.41; N, 18.13%.

Preparation 13

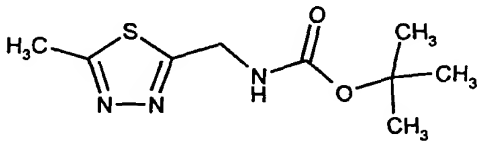
Benzyl 3-(methylamino)-3-oxopropylcarbamate



A mixture of N-[(benzyloxy)carbonyl]-β-alanine (10g, 44.8mmol), methylamine hydrochloride (3.33g, 49.28mmol), 1-hydroxybenzotriazole hydrate (6.05g, 44.8mmol), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (10.3g, 53.76mmol) and N-methylmorpholine (11.33ml, 103mmol) in dichloromethane (200ml) was stirred at room temperature for 18 hours. The resulting precipitate was filtered off to give the desired product as a colourless foam, and the filtrate evaporated under reduced pressure. The residue was purified by column chromatography on silica gel using an elution gradient of ethyl acetate:hexane (90:10 to 100:0) to give additional product, 7.96g, 75% in total; ^{1}H NMR ($CDCl_3$, 300MHz) δ: 2.42 (t, 2H), 2.80 (s, 3H), 3.50 (m, 2H), 5.21 (s, 2H), 5.49 (bs, 1H), 5.63 (bs, 1H), 7.36 (m, 5H); Anal. Found: C, 60.68; H, 7.00; N, 11.95. $C_{12}H_{16}N_2O_3$ requires C, 61.00; H, 6.83; N, 11.86%.

Preparation 14

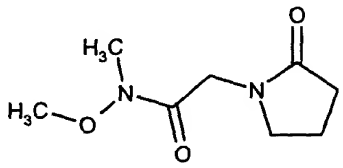
tert-Butyl (5-methyl-1,3,4-thiadiazol-2-yl)methylcarbamate



Lawesson's reagent (960mg, 2.38mmol) was added to a solution of the hydrazide from preparation 12 (500mg, 2.16mmol) in tetrahydrofuran (40ml), and the reaction heated under reflux for 3 hours, then stirred at room temperature for 18 hours. The mixture was evaporated under reduced pressure and the residue purified by column chromatography on silica gel using an elution gradient of ethyl acetate:pentane (70:30 to 80:20) to give an oil. This was dissolved in ethyl acetate (100ml), charcoal (2g) added, the mixture stirred for 10 minutes then filtered. The filtrate was concentrated under reduced pressure, and the residue azeotroped with dichloromethane to afford the title compound as a crystalline solid, 441mg, 89%; ^{1}H NMR ($CDCl_3$, 400MHz) δ: 1.45 (s, 9H), 2.77 (s, 3H), 4.66 (d, 2H), 5.22 (bs, 1H); LRMS : m/z 230.1 (MH^+).

Preparation 15

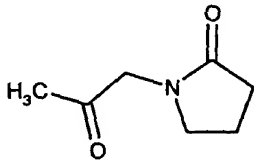
N-Methoxy-*N*-methyl-2-(2-oxo-1-pyrrolidinyl)acetamide



2-Chloro-N-methoxy-N-methylacetamide (3.2g, 23.3mmol) was added to a suspension of 2-pyrrolidinone (2.0g, 23.5mmol) and sodium hydride (940mg, 60% dispersion in mineral oil, 23.5mmol) in tetrahydrofuran (60ml), and the reaction stirred at room temperature for 48 hours. The mixture was quenched with water (150ml), and extracted with ethyl acetate (200ml) and dichloromethane (200ml). The combined organic extracts were dried ($MgSO_4$) and evaporated under reduced pressure. The residue was triturated with hexane, then ether to afford the title compound as white crystals, 1.8g, 41%; ^1H NMR ($CDCl_3$, 400MHz) δ: 2.02 (m, 2H), 2.40 (t, 2H), 3.17 (s, 3H), 3.48 (t, 2H), 3.72 (s, 3H), 4.19 (s, 2H); LRMS : m/z 186.9 (MH^+).

Preparation 16

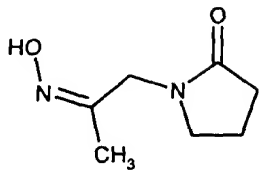
1-(2-Oxopropyl)-2-pyrrolidinone



Methylmagnesium chloride (2.7ml, 3M in tetrahydrofuran, 8.1mmol) was added to a cooled (-20°C) solution of the amide from preparation 15 (1.5g, 8.1mmol) in tetrahydrofuran (50ml), and the reaction allowed to warm to room temperature, then stirred for an hour. The mixture was quenched by the addition of aqueous ammonium chloride solution, then extracted with ethyl acetate (3x50ml). The combined organic solutions were dried ($MgSO_4$), and evaporated under reduced pressure to give the title compound as an oil, 645mg, 56%; ^1H NMR ($CDCl_3$, 400MHz) δ: 2.07 (m, 2H), 2.17 (s, 3H), 2.42 (t, 2H), 3.42 (t, 2H), 4.10 (s, 2H).

Preparation 17

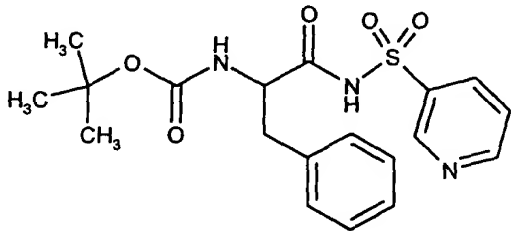
1-[2-(Hydroxyimino)propyl]-2-pyrrolidinone



Hydroxylamine hydrochloride (316mg, 4.55mmol) and then pyridine (370µl, 4.58mmol) were added to a solution of the amide from preparation 16 (643mg, 4.55mmol) in ethanol (30ml), and the reaction stirred at room temperature for 18 hours. The mixture was evaporated under reduced pressure and the residue purified by column chromatography on silica gel using an elution gradient of dichloromethane:methanol (97:3 to 90:10). The product was triturated with ether to give the title compound as a white solid, 375mg, 53%; ^{1}Hmr (DMSOd_6, 400MHz) δ: 1.60 (s, 3H), 1.87 (m, 2H), 2.20 (t, 2H), 3.19 (t, 2H), 3.78 (s, 2H), 10.77 (s, 1H); LRMS : m/z 157.4 (MH^+).

Preparation 18

tert-Butyl 1-benzyl-2-oxo-2-[(3-pyridinylsulfonyl)amino]ethylcarbamate

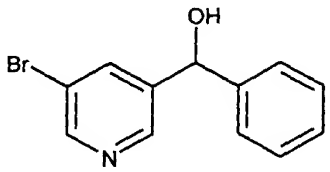


1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (939mg, 4.9mmol), 1-hydroxybenzotriazole hydrate (562mg, 4.15mmol), and N-methylmorpholine (952mg, 9.42mmol) were added to an ice-cold solution of N-*tert*-butoxycarbonyl-L-phenylalanine (1.0g, 3.77mmol) in dichloromethane (20ml), and the mixture stirred for 15 minutes. 3-Pyridinesulphonamide (Mon. für Chemie; 72; 77; 1938) (596mg, 3.77mmol) was added, and the reaction stirred at room temperature for 24 hours. The mixture was evaporated under reduced pressure and the residue partitioned between ethyl acetate (50ml) and water (50ml), and the layers separated. The aqueous layer was extracted well with ethyl acetate, then dichloromethane, the combined organic extracts dried ($MgSO_4$) and evaporated under reduced pressure. The crude product was purified twice by column chromatography on silica gel, using an elution gradient of ethyl acetate:ethanol (100:0 to 90:10) to give the desired product as a white foam, 1.01g, 66; ^{1}H NMR (DMSOd_6, 300MHz) δ: 1.30 (s, 9H), 2.77 (m, 1H), 2.97 (m, 1H),

3.84 (m, 1H), 5.95 (bs, 1H), 6.96 (m, 2H), 7.08 (m, 3H), 7.42 (m, 1H), 8.05 (d, 1H), 8.60 (d, 1H), 8.84 (m, 1H); $[\alpha]_D$ = -10° (0.1% solution in methanol).

Preparation 19

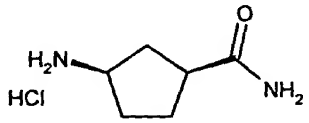
(5-Bromo-3-pyridinyl)(phenyl)methanol



n-Butyl lithium (17ml, 2.5M in hexanes, 42.5mmol) was added dropwise to cooled (-78 °C) solution of 3,5-dibromopyridine (10g, 42.2mmol) in ether (200ml), so as to maintain an internal temperature <-70°C. The mixture was then stirred for 15 minutes, and a solution of benzaldehyde (4.5g, 42.5mmol) in ether (20ml) was added dropwise, again maintaining the temperature <-70°C. The mixture was stirred for 15 minutes, then allowed to warm to room temperature over an hour. The reaction was quenched by the addition of 0.9M ammonium chloride solution (200ml), the layers separated, and the aqueous phase extracted with ether. The combined organic extracts were dried ($MgSO_4$) and evaporated under reduced pressure. The residual yellow oil was purified by column chromatography on silica gel using an elution gradient of dichloromethane:ether (95:5 to 80:20) to give the title compound as a yellow oil, 7.6g, 68%; ^{1}H NMR (D_2O, 300MHz) δ: 5.80 (s, 1H), 7.37 (m, 5H), 7.90 (s, 1H), 8.40 (s, 1H), 8.44 (s, 1H).

Preparation 20

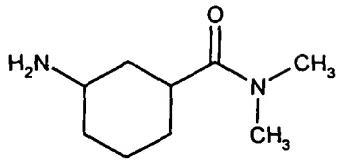
(1*S*,3*R*)-3-Aminocyclopentanecarboxamide hydrochloride



Hydrogen chloride gas was bubbled through an ice-cooled solution of the amide from preparation 10 (438mg, 1.92mmol) in dichloromethane (50ml) for 10 minutes, and the resulting suspension stirred at room temperature for 2 hours. The mixture was purged with nitrogen, then evaporated under reduced pressure. The residue was triturated with ether, to afford the title compound as a solid; ^{1}H NMR (D_2O, 400MHz) δ: 1.63-1.82 (m, 3H), 1.92-2.07 (m, 2H), 2.19 (m, 1H), 2.82 (m, 1H), 3.62 (m, 1H).

Preparation 21

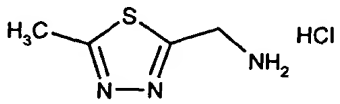
3-Amino-*N,N*-dimethylcyclohexanecarboxamide



A solution of the amide from preparation 11 (997mg, 3.69mmol) in trifluoroacetic acid (8ml) and dichloromethane (8ml) was stirred at room temperature for 4 hours. The mixture was concentrated under reduced pressure and the residue partitioned between dichloromethane (25ml) and sodium bicarbonate solution (25ml). The pH was adjusted to 9 using sodium hydroxide solution, the layers separated, and the aqueous phase evaporated under reduced pressure. The resulting solid was triturated with hot ethyl acetate, the suspension filtered and the filtrate concentrated under reduced pressure. The crude product was purified by column chromatography on silica gel using dichloromethane:methanol:0.88 ammonia (84:14:2) to afford the title compound as a colourless oil, 346mg, 55%; ^{1}H NMR ($CDCl_3$, 300MHz) δ: 1.08 (m, 1H), 1.25-1.54 (m, 6H), 1.72 (m, 1H), 1.86 (m, 2H), 2.53-2.75 (m, 2H), 2.96 (s, 3H), 3.03 (s, 3H);

Preparation 22

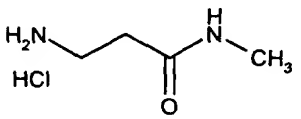
(5-Methyl-1,3,4-thiadiazol-2-yl)methylamine hydrochloride



Hydrogen chloride gas was bubbled through an ice-cooled solution of the thiadiazole from preparation 14 (425mg, 1.85mmol) in dichloromethane (50ml) for 15 minutes, and the reaction stirred at room temperature for 1 hour. The mixture was purged with nitrogen, then evaporated under reduced pressure to afford the title compound as a white solid; ^{1}H NMR ($DMSOd_6$, 400MHz) δ: 2.75 (s, 3H), 4.48 (m, 2H), 8.80 (bs, 3H).

Preparation 23

3-Amino-N-methylpropanamide hydrochloride

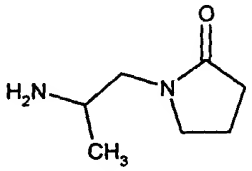


A mixture of the benzyl carbamate from preparation 13 (7.92g, 33.5mmol) and 5% palladium on charcoal (800mg) in ethanol (300ml) was hydrogenated at 50 psi and

room temperature for 4 hours. The reaction mixture was filtered through Arbocel®, washing through with ethanol, and 1N hydrochloric acid (36.9ml, 36.9mmol) was added to the combined filtrate. This solution was evaporated under reduced pressure and the residue azeotroped with dichloromethane to afford the title compound as a colourless foam, 4.66g, ^{1}H NMR ($DMSOd_6$, 300MHz) δ: 2.46 (t, 2H), 2.60 (s, 3H), 2.95 (m, 2H), 7.98-8.16 (m, 2H).

Preparation 24

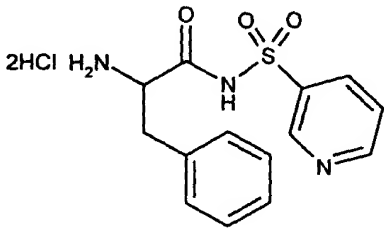
1-(2-Aminopropyl)-2-pyrrolidinone



A mixture of the oxime from preparation 17 (375mg, 2.40mmol) and platinum oxide (300mg) in ethanol (20ml) was hydrogenated at 60psi and room temperature for 18 hours. Tlc analysis showed starting material remaining, so additional platinum oxide (100mg) was added and the reaction continued for a further 4 hours. The mixture was filtered through Arbocel®, and the filtrate evaporated under reduced pressure. The crude product was purified by column chromatography on silica gel using an elution gradient of dichloromethane:methanol:0.88 ammonia (95:5:0.5 to 90:10:1) to give the title compound as a clear oil, 170mg, 50%; ^{1}H NMR ($CDCl_3$, 400MHz) δ: 1.02 (d, 3H), 1.36 (bs, 2H), 2.00 (m, 2H), 2.38 (t, 2H), 3.00-3.16 (m, 2H), 3.21 (m, 1H), 3.35-3.45 (m, 2H); LRMS : m/z 143 (MH^+).

Preparation 25

N-(2-Amino-3-phenylpropanoyl)-3-pyridinesulphonamide dihydrochloride

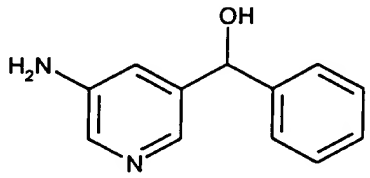


Saturated ethereal hydrochloric acid (40ml) was added to an ice-cold solution of the sulphonamide from preparation 18 (959mg, 2.37mmol) in ethyl acetate (30ml) and ether (10ml), and the solution stirred at room temperature for 18 hours. The reaction mixture was concentrated under reduced pressure and the residue azeotroped with

dichloromethane (3x) to afford the title compound as a white solid, 959mg; ^{1}H NMR ($DMSOd_6$, 300MHz) δ: 3.23-3.50 (m, 1H), 3.70-3.98 (m, 1H), 4.13 (m, 1H), 7.05 (m, 2H), 7.20 (m, 3H), 7.78 (m, 1H), 8.36 (d, 1H), 8.44 (bs, 2H), 8.95 (d, 1H), 9.02 (s, 1H);[$\alpha]_D$ = +138° (0.5% solution in methanol).

Preparation 26

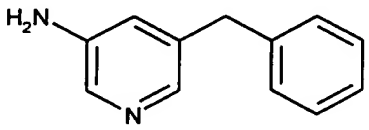
(5-Amino-3-pyridinyl)(phenyl)methanol



A mixture of the bromide from preparation 19 (2.0g, 7.60mmol) and copper (II) sulphate pentahydrate (350mg, 1.40mmol) in 0.88 ammonia (18ml) was heated at 135 °C in a sealed vessel for 24 hours. Sodium hydroxide solution (1N, 10ml) was added to the cooled solution, and the mixture was then extracted with ether (6x). The combined organic extracts were dried ($MgSO_4$), and concentrated under reduced pressure to a low volume. The resulting precipitate was filtered, washed with ether and dried to give the title compound as a solid, 1.25g, 83%; mp 92-94°C; ^{1}H NMR ($DMSOd_6$, 300MHz) δ: 5.22 (s, 2H), 5.59 (d, 1H), 5.86 (d, 1H), 6.83 (s, 1H), 7.20 (m, 1H), 7.34 (m, 4H), 7.78 (m, 2H).

Preparation 27

5-Benzyl-3-pyridinylamine

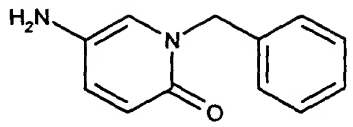


A mixture of the alcohol from preparation 26 (700mg, 3.5mmol) and 5% palladium on charcoal (70mg) in hydrochloric acid (5ml, 1N) and ethanol (20ml) was hydrogenated at 30 psi and room temperature for 6 hours. The mixture was filtered through Arbocel ®, and the filtrate concentrated under reduced pressure. The residue was basified using aqueous sodium bicarbonate solution, extracted with dichloromethane (3x), and the combined organic extracts dried ($MgSO_4$), and evaporated under reduced pressure. The crude product was purified by column chromatography on silica gel using dichloromethane:methanol:0.88 ammonia (92:8:0.4) as eluant, to give the title compound as a solid, 500mg, 78%; mp 107-109°C; ^{1}H NMR ($CDCl_3$, 300MHz) δ: 3.61

(bs, 2H), 3.94 (s, 2H), 6.78 (s, 1H), 7.24 (m, 5H), 7.98 (s, 2H).

Preparation 28

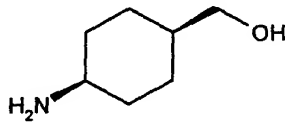
5-Amino-1-benzyl-2(1*H*)-pyridinone



A mixture of 1-benzyl-5-nitro-1H-pyridin-2-one (Justus Liebigs Ann. Chem. 484; 1930; 52) (1.0g, 4.35mmol), and granulated tin (3.5g, 29.5mmol) in concentrated hydrochloric acid (14ml) was heated at 90°C for 1.5 hours. The cooled solution was diluted with water, neutralised using sodium carbonate solution, and extracted with ethyl acetate (250ml in total). The combined organic extracts were filtered, dried ($MgSO_4$), and evaporated under reduced pressure to give the title compound as a pale green solid, (turned blue with time), 440mg, 51%; ^{1}H NMR ($CDCl_3$, 250MHz) δ: 4.12-4.47 (bs, 2H), 5.00 (s, 2H), 6.31 (d, 1H), 6.86 (s, 1H), 7.07 (m, 1H), 7.14-7.42 (m, 5H).

Preparation 29

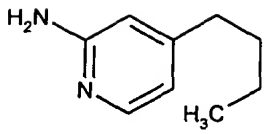
Cis-(4-Aminocyclohexyl)methanol



Lithium aluminium hydride (14ml, 1M solution in tetrahydrofuran, 14mmol) was added dropwise to an ice-cooled solution of cis-4-aminocyclohexanecarboxylic acid (1.33g, 9.29mmol) in tetrahydrofuran (50ml), and once addition was complete, the reaction was heated under reflux for 6 hours. The resulting suspension was cooled to 5°C, and water (0.6ml), aqueous sodium hydroxide solution (1.1ml, 2M), then water (0.6ml) were added sequentially. The resulting suspension was filtered, and the filtrate evaporated under reduced pressure to give an oil, which was used without further purification; ^{1}H NMR ($CDCl_3$, 300MHz) δ: 1.40-1.80 (m, 12H), 3.00 (m, 1H), 3.55 (d, 2H); LRMS : m/z 130.2 (MH^+).

Preparation 30

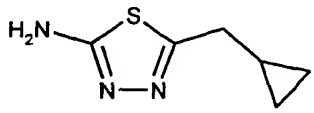
2-Amino-4-butylpyridine



A mixture of 4-butylpyridine (5.0g, 37.0mmol) and 95% sodium amide (1.7g, 40.7mmol) in xylene (10ml) was heated at 150°C for 18 hours. The cooled mixture was diluted with ether (100ml) and extracted with 2N hydrochloric acid (twice). The aqueous extracts were basified using sodium hydroxide solution, and re-extracted with ether. These combined organic extracts were dried ($MgSO_4$) and evaporated under reduced pressure. The residual oil was purified by column chromatography on silica gel using dichloromethane:methanol:0.88 ammonia (97:3:0.15) as eluant, to afford the title compound as a crystalline solid, 2.1g, 38%; ^{1}H NMR ($CDCl_3$, 300MHz) δ: 0.96 (t, 3H), 1.38 (m, 2H), 1.60 (m, 2H), 2.52 (t, 2H), 4.38 (bs, 2H), 6.38 (s, 1H), 6.55 (d, 1H), 7.98 (d, 1H); Anal. Found: C, 72.01; H, 9.47; N, 18.53. $C_9H_{14}N_2$ requires C, 71.96; H, 9.39; N, 18.65%.

Preparation 31

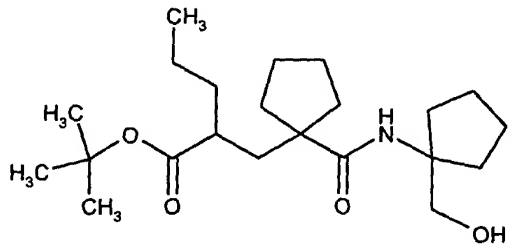
5-(Cyclopropylmethyl)-1,3,4-thiadiazol-2-amine



Oxalyl chloride (3.13ml, 35.9mmol) and N,N-dimethylformamide (1 drop) were added to a solution of cyclopropylacetic acid (3g, 29.9mmol) in dichloromethane (30ml), and the reaction stirred at room temperature for 18 hours. The mixture was concentrated under reduced pressure and azeotroped with dichloromethane to give a brown oil.
A mixture of this intermediate acid chloride (887mg, 7.48mmol) and thiosemicarbazide (455mg, 4.99mmol) were heated at 70°C for 18 hours, then cooled. Water was added, the mixture basified to pH 9 using 50% aqueous sodium hydroxide solution, and the resulting precipitate filtered and dried, to give a cream solid, 410mg, 53%; ^{1}H NMR (CD_3OD, 400MHz) δ: 0.28 (m, 2H), 0.60 (m, 2H), 1.02 (m, 1H), 2.77 (d, 2H); LRMS : m/z 155.2 (MH^+).

Preparation 33

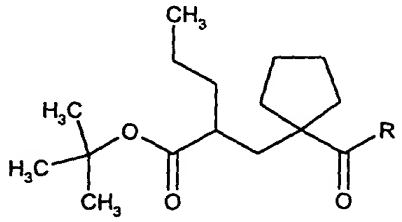
tert-Butyl 2-{[1-({[1-(hydroxymethyl)cyclopentyl]amino}carbonyl)-cyclopentyl]methyl}pentanoate



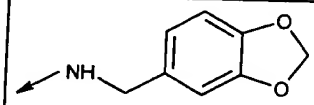
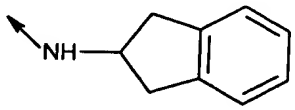
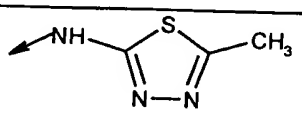
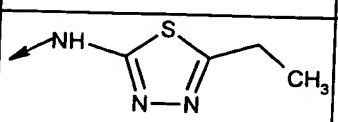
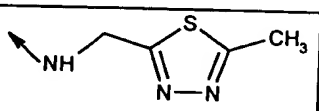
1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (41mg, 0.21mmol), 1-hydroxybenzotriazole hydrate (27mg, 0.2mmol), N-methylmorpholine (35µl, 0.31mmol) and finally 1-amino-1-cyclopentanemethanol (25mg, 0.22mmol) were added to a solution of the acid from preparation 1 (150mg, 0.53mmol) in N,N-dimethylformamide (3ml), and the reaction stirred at 90°C for 18 hours. The cooled solution was diluted with ethyl acetate (90ml), washed with water (3x25ml), and brine (25ml), then dried ($MgSO_4$) and evaporated under reduced pressure. The crude product was purified by chromatography on silica gel, using ethyl acetate:pentane (30:70) as the eluant to afford the title compound, 38mg, 57%; ^{1}H NMR ($CDCl_3$, 400MHz) δ: 0.88 (t, 3H), 1.29 (m, 3H), 1.41-1.78 (m, 26H), 1.78-1.98 (m, 4H), 2.04 (m, 1H), 2.26 (m, 1H), 3.59 (dd, 1H), 3.70 (dd, 1H), 4.80 (t, 1H), 5.81 (s, 1H); LRMS : m/z 380 (MH^-).

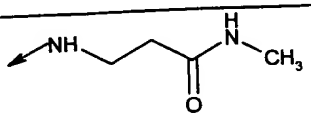
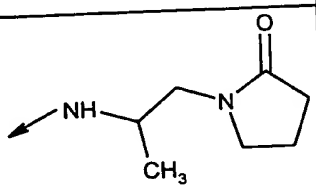
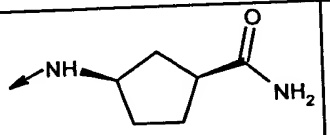
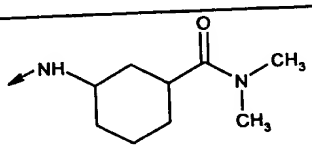
Preparations 34 to 43

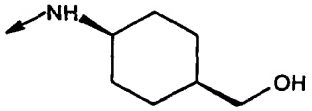
The following compounds of general structure:



were prepared from the acid from preparation 1 and the appropriate amine compound, following a similar procedure to that described in preparation 33.

Prep	R	Starting amine	Yield (%)	Data
34		Piperonylamine	88	^{1}H NMR ($CDCl_3$, 400MHz) δ: 0.85 (t, 3H), 1.26 (m, 4H), 1.42 (s, 9H), 1.46 (m, 2H), 1.59-1.75 (m, 5H), 1.95 (m, 2H), 2.06 (m, 1H), 2.22 (m, 1H), 4.26 (dd, 1H), 4.39 (dd, 1H), 5.95 (m, 3H), 6.78 (m, 3H). LRMS : m/z 418.3 (MH^+)
35[1]		2-Aminoindan hydrochloride	40	^{1}H NMR ($CDCl_3$, 400MHz) δ: 0.87 (t, 3H), 1.25 (m, 3H), 4H), 1.42 (m, 12H), 1.56-1.70 (m, 4H), 1.90 (m, 2H), 2.02 (m, 1H), 2.22 (m, 1H), 2.80 (m, 2H), 3.35 (m, 2H), 4.76 (m, 1H), 5.86 (d, 1H), 7.19 (m, 4H). LRMS : m/z 400.3 (MH^+)
36[2]		2-Amino-5-methyl-1,3,4-thiadiazole	76	^{1}H NMR ($CDCl_3$, 400MHz) δ: 0.82 (t, 3H), 1.20-1.85 (m, 20H), 2.18 (m, 4H), 2.67 (s, 3H), 9.80 (bs, 1H). LRMS : m/z 382.3 (MH^+)
37[2]		2-Amino-5-ethyl-1,3,4-thiadiazole	92	^{1}H NMR ($CDCl_3$, 300MHz) δ: 0.82 (t, 3H), 1.20-1.80 (m, 22H), 1.84 (m, 1H), 2.20 (m, 4H), 3.04 (q, 2H), 9.10 (bs, 1H). LRMS : m/z 396.2 (MH^+)
38		Preparation 22	77	^{1}H NMR ($CDCl_3$, 300MHz) δ: 0.84 (t, 3H), 1.20-1.38 (m, 4H), 1.42 (s, 9H), 1.44-1.76 (m, 7H), 1.95-2.12 (m, 3H), 2.20 (m, 1H), 2.76 (s, 3H), 4.74 (dd, 1H), 4.82 (dd, 1H), 6.54 (bs, 1H). LRMS : m/z 396.2 (MH^+)

Prep	R	Starting amine	Yield (%)	Data
39[1,2]		Preparation 23	60	^{1}H NMR ($CDCl_3$, 300MHz) δ: 0.88 (t, 3H), 1.21-1.38 (m, 3H), 1.40-1.70 (m, 17H), 1.88-2.04 (m, 3H), 2.20 (m, 1H), 2.39 (t, 2H), 2.80 (d, 3H), 3.53 (m, 2H), 6.13 (bs, 1H), 6.40 (m, 1H). LRMS : m/z 369.5 (MH^+)
40[2]		Preparation 24	70	^{1}H NMR ($CDCl_3$, 300MHz) δ: 0.82 (m, 3H), 1.16 (2xd, 3H), 1.20-1.72 (m, 21H), 1.83 (m, 1H), 1.98 (m, 3H), 2.17 (m, 1H), 2.38 (m, 2H), 1.96 (m, 1H), 3.34 (m, 1H), 3.54-3.62 (m, 2H), 4.15-4.20 (m, 1H), 6.21-6.35 (2xbd, 1H). LRMS : m/z 409.3 (MH^+).
41[2]		Preparation 20	94	^{1}H NMR ($CDCl_3$, 400MHz) δ: 0.82 (t, 3H), 1.19-1.38 (m, 4H), 1.42 (m, 12H), 1.60 (m, 3H), 1.74-2.02 (m, 10H), 2.18 (m, 1H), 2.78 (m, 1H), 4.38 (m, 1H), 5.32 (bs, 1H), 5.57 (bs, 1H), 7.28 (bs, 1H). LRMS : m/z 395 (MH^+)
42[2]		Preparation 21	91	^{1}H NMR ($CDCl_3$, 300MHz) δ: 0.86 (t, 3H), 1.18-1.78 (m, 25H), 1.84-2.03 (m, 6H), 2.22 (m, 1H), 2.68 (m, 1H), 2.96 (s, 3H), 3.03 (s, 3H), 3.84 (m, 1H), 5.78 (m, 1H). LRMS : m/z 437.7 (MH^+)

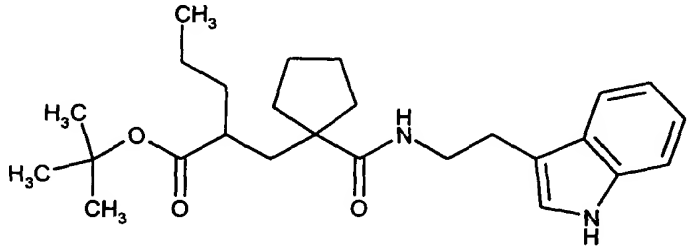
Prep	R	Starting amine	Yield (%)	Data
43[2]		Preparation 29	99	^{1}H NMR ($CDCl_3$, 300MHz) δ: 0.85 (t, 3H), 1.20-1.79 (m, 30H), 1.90 (m, 2H), 2.05 (m, 1H), 2.24 (m, 1H), 3.56 (m, 2H), 4.04 (m, 1H), 5.82 (bd, 1H). LRMS : m/z 396.4 (MH^+)

1 = reaction conducted at room temperature

2 = Methanol:dichloromethane was used as the column eluant

Preparation 44

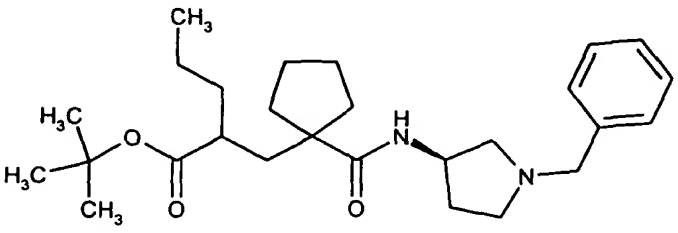
tert-Butyl 2-{[1-({[2-(1*H*-indol-3-yl)ethyl]amino}carbonyl)cyclopentyl]methyl}pentanoate



The title compound was obtained as a pale yellow oil in 80% yield from the acid from preparation 1 and tryptamine, following a similar procedure to that described in preparation 33, except the reaction was performed in dichloromethane at room temperature; ^{1}H NMR ($CDCl_3$, 400MHz) δ: 0.86 (t, 3H), 1.26 (m, 3H), 1.42 (m, 11H), 1.50-1.69 (m, 6H), 1.83 (m, 1H), 1.90-2.05 (m, 2H), 2.22 (m, 1H), 2.99 (t, 3H), 3.60 (m, 2H), 5.78 (m, 1H), 7.06 (s, 1H), 7.14 (m, 1H), 7.20 (m, 1H), 7.38 (d, 1H), 7.63 (d, 1H), 8.02 (bs, 1H); LRMS : m/z 427.5 (MH^+).

Preparation 45

tert-Butyl 2-[(1-{[(3*S*)-1-benzylpyrrolidinyl]amino}cyclopentyl)methyl]pentanoate

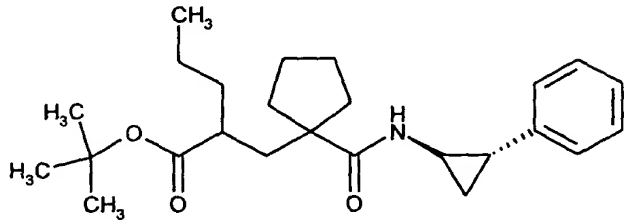


The title compound was obtained quantitatively from the acid from preparation 1 and (3S)-1-benzyl-3-aminopyrrolidine, following a similar procedure to that described in

preparation 44; ^{1}H NMR ($CDCl_3$, 300MHz) δ: 0.84 (t, 3H), 1.10-1.76 (m, 21H), 1.90-2.05 (m, 3H), 2.20-2.38 (m, 3H), 2.58 (m, 2H), 2.84 (m, 1H), 3.62 (s, 2H), 4.45 (m, 1H), 6.02 (m, 1H), 7.33 (m, 5H).

Preparation 46

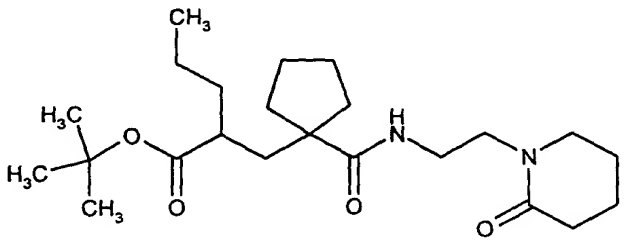
tert-Butyl 2-{[1-({[cis-2-phenylcyclopropyl]amino}carbonyl)-cyclopentyl]methyl}pentanoate



1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (81mg, 0.42mmol), N-methylmorpholine (0.15ml, 1.06mmol) and tranylcypromine hydrochloride (60mg, 0.35mmol) were added to a solution of the acid from preparation 1 (100mg, 0.35mmol) in dichloromethane (10ml), and the reaction stirred at room temperature for 18 hours. The reaction mixture was evaporated under reduced pressure and the residue purified by column chromatography on silica gel using an elution gradient of dichloromethane:methanol (98:2 to 95:5) to afford the title compound as a yellow oil, 85mg, 55%; ^{1}H NMR ($CDCl_3$, 300MHz) δ: 0.88 (t, 3H), 1.16 (m, 1H), 1.20-1.58 (m, 16H), 1.63 (m, 5H), 1.90-2.14 (m, 4H), 2.23 (m, 1H), 2.90 (m, 1H), 6.00 (m, 1H), 7.19 (m, 3H), 7.24 (m, 2H); LRMS : m/z 400 (MH^+).

Preparation 47

tert-Butyl 2-{[1-({[2-(2-oxo-1-piperidinyl)ethyl]amino}carbonyl)cyclopentyl]-methyl}pentanoate

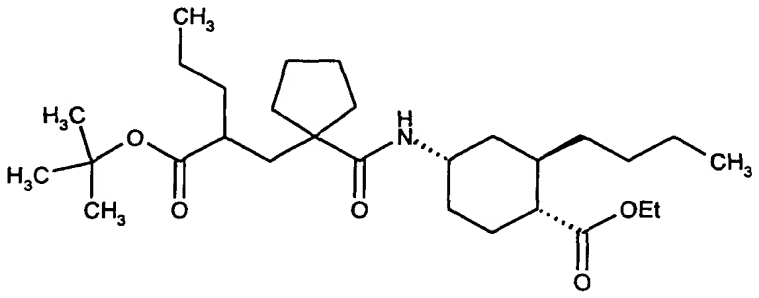


Hydrazine monohydrate (34μl, 0.70mmol) was added to a solution of the compound from preparation 6 (171mg, 0.63mmol) in ethanol (10ml), and the reaction heated under reflux for 5 hours. The cooled mixture was filtered, the filtrate concentrated under reduced pressure, the residue suspended in dichloromethane, and the

suspension re-filtered. The resulting filtrate was concentrated under reduced pressure, and the residue purified by column chromatography on silica gel using dichloromethane:methanol:0.88 ammonia (90:10:1) as eluant to give the amine, 16mg. The acid from preparation 1 (32mg, 0.11mmol), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (25mg, 0.13mmol), 1-hydroxybenzotriazole hydrate (17mg, 0.13mmol), and N-methylmorpholine (25µl, 0.23mmol) were added to a solution of this amine in N,N-dimethylformamide (2ml), and the reaction stirred at room temperature for 18 hours. The mixture was partitioned between ethyl acetate and water, and the layers separated. The organc phase was washed with water (2x), dried ($MgSO_4$), and evaporated under reduced pressure. The residual oil was purified by column chromatography on silica gel using an elution gradient of dichloromethane:methanol (98.5:1.5 to 95:5) to afford the title compound as an oil, 43mg, 17%; ^1H NMR ($CDCl_3$, 400MHz) δ: 0.82 (t, 3H), 1.22 (m, 3H), 1.38-1.65 (m, 17H), 1.58 (m, 4H), 1.95 (m, 3H), 2.17 (m, 1H), 2.37 (m, 2H), 3.30 (m, 2H), 3.38 (m, 2H), 3.50 (m, 2H), 6.76 (m, 1H); LRMS : m/z 409.2 (MH^+)

Preparation 48

Ethyl (1*R*,2*R*,4*S*)-4-[({1-[2-(*tert*-butoxycarbonyl)pentyl]cyclopentyl}carbonyl)amino]-2-butylcyclohexanecarboxylate

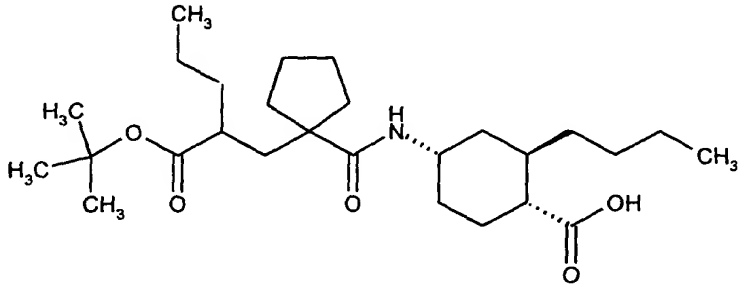


A mixture of the acid from preparation 1 (109mg, 0.38mmol), (1*R*,2*R*,4*S*)-4-amino-2-butyl-cyclohexanecarboxylic acid ethyl ester hydrochoride (WO, 9009374), (101mg, 0.38mmol), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (95mg, 0.50mmol), 1-hydroxybenzotriazole hydrate (60mg, 0.40mmol) and triethylamine (0.12ml, 0.87mmol) in dichloromethane (3ml), was stirred at room temperature for 16 hours. The mixture was evaporated under reduced pressure, the residue treated with sodium bicarbonate solution and extracted with ethyl acetate. The combined organic extracts were dried ($MgSO_4$) and evaporated under reduced pressure to give a gum. The crude product was purified by column chromatography on silica gel using ethyl acetate:pentane (50:50) as eluant, and azeotroped with dichloromethane to afford the

title compound, 190mg; ^{1}H NMR ($CDCl_3$, 300MHz) δ: 0.88 (m, 6H), 1.20-1.40 (m, 13H), 1.40-2.10 (m, 25H), 2.16-2.30 (m, 2H), 4.18 (m, 3H), 5.83 (d, 1H).

Preparation 49

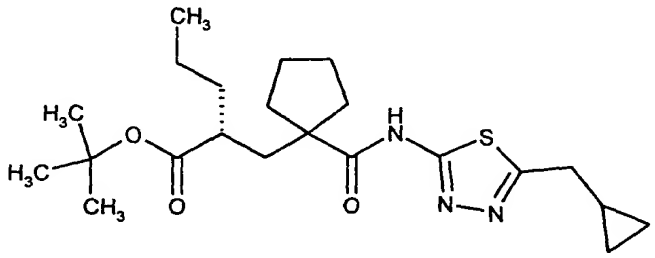
(1*R*, 2*R*,4*S*)-4-[({1-[2-(*tert*-Butoxycarbonyl)pentyl]cyclopentyl}carbonyl)amino]-2-butylcyclohexanecarboxylic acid



A mixture of the ethyl ester from preparation 48 (190mg, 0.39mmol) and 1N sodium hydroxide solution (0.85ml, 0.85mmol) in methanol (1.5ml) was stirred at room temperature for 22 hours. The reaction mixture was acidifed to pH 1 using hydrochloric acid (2N), then partitioned between ethyl acetate and water. The layers were separated, and the organic phase was dried ($MgSO_4$) and evaporated under reduced pressure to afford the title compound, 130mg, 72%; ^{1}H NMR ($CDCl_3$, 300MHz) δ: 0.86 (m, 6H), 1.20-2.12 (m, 36H), 2.24 (m, 2H), 4.18 (m, 1H), 5.82 (d, 1H); LRMS : m/z 464 $(M-H)^-$

Preparation 50

tert-Butyl (2*R*)-2-[[1-({[5-(cyclopropylmethyl)-1,3,4-thiadiazol-2-yl]amino}carbonyl)cyclopentyl]methyl}pentanoate

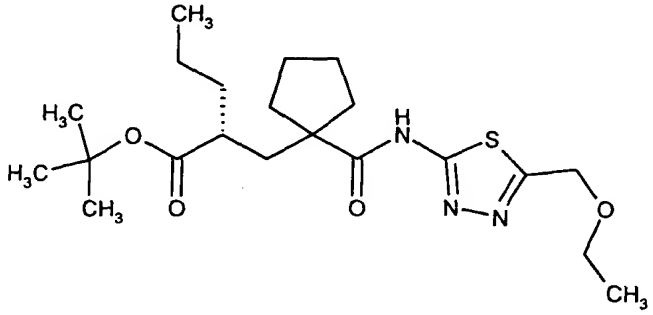


The title compound was prepared from the acid from preparation 2 and the amine from preparation 31, in 65% yield, following the procedure described in preparation 33; ^{1}H NMR ($CDCl_3$, 400MHz) δ: 0.35 (m, 2H), 0.63 (m, 2H), 0.80 (m, 3H), 1.10 (m, 1H), 1.20-1.94 (m, 20H), 2.19 (m, 4H), 2.93 (t, 2H), 3.50 (s, 1H); LRMS : m/z 422.4 (MH^+)

$[\alpha]_D$ = -14.15° (c = 0.082, methanol).

Preparation 51

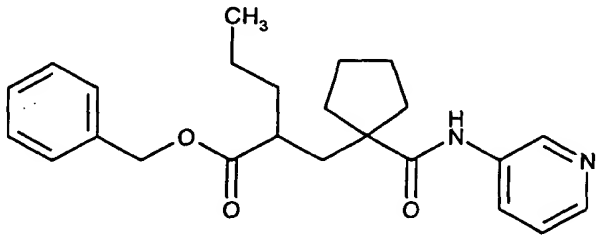
tert-Butyl (2*R*)-2-{[1-({[5-(ethoxymethyl)-1,3,4-thiadiazol-2-yl]amino}carbonyl)-cyclopentyl]methyl}pentanoate



The title compound was prepared from the acid from preparation 2 and 5-(ethoxymethyl)-1,3,4-thiadiazol-2-amine, in 51% yield, following the procedure described in preparation 33; ^{1}H NMR ($CDCl_3$, 400MHz) δ: 1.10-1.78 (m, 25H), 1.82 (m, 1H), 2.19 (m, 5H), 3.48 (s, 1H), 4.82 (s, 2H), 10.16 (brs, 1H); LRMS : m/z 426.4 (MH^+); $[\alpha]_D$ = -12.50° (c = 0.08, methanol).

Preparation 52

Benzyl 2-({1-[(3-pyridinylamino)carbonyl]cyclopentyl}methyl)pentanoate

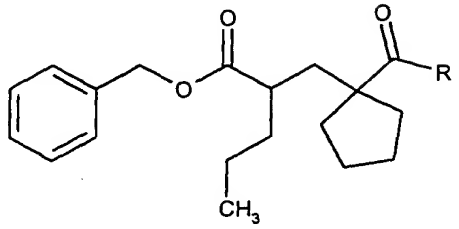


Triethylamine (0.11ml, 0.78mmol) was added to a mixture of the acid chloride from preparation 3 (200mg, 0.60mmol) and 2-aminopyridine (61mg, 0.65mmol) in dichloromethane (3ml), and the reaction stirred at room temperature for 16 hours. The mixture was evaporated under reduced pressure, the residue partitioned between sodium bicarbonate solution (5ml) and ethyl acetate (20ml), and the layers separated. The organic phase was dried ($MgSO_4$), and evaporated under reduced pressure to give a gum. The crude product was purified by column chromatography on silica gel using ethyl acetate as eluant, to afford the title compound, 130mg; ^{1}H NMR ($CDCl_3$, 400MHz) δ: 0.82 (t, 3H), 1.21 (m, 3H), 1.40 (m, 1H), 1.43-1.72 (m, 6H), 1.81 (d, 1H), 1.98 (m, 1H), 2.18 (m, 1H), 2.24 (m, 1H), 2.46 (m, 1H), 4.98 (m, 2H), 7.20-7.38 (m,

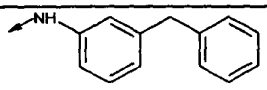
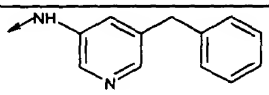
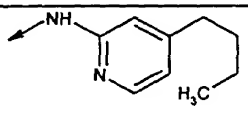
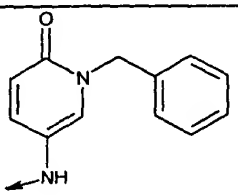
6H), 7.42 (s, 1H), 8.06 (d, 1H), 8.35 (d, 1H), 8.56 (s, 1H).

Preparations 53 to 56

The following compounds of general formula:



were prepared from the acid chloride from preparation 3 and the appropriate amine, following a similar procedure to that described in preparation 52.

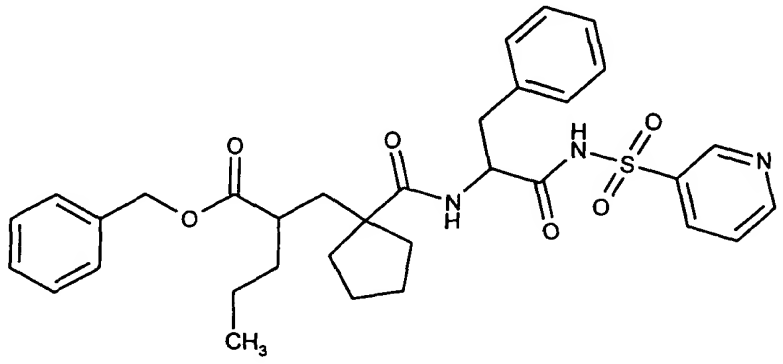
Prep	R	Yield (%)	Data
53[1]		90	^{1}H NMR ($CDCl_3$, 300MHz) δ: 0.84 (t, 3H), 1.24 (m, 2H), 1.40-1.76 (m, 7H), 1.84 (dd, 1H), 1.98 (m, 1H), 2.19 (dd, 1H), 2.28 (m, 1H), 2.56 (m, 1H), 3.98 (s, 2H), 4.99 (dd, 2H), 6.98 (d, 1H), 7.19-7.42 (m, 15H).
54		65	^{1}H NMR ($CDCl_3$, 300MHz) δ: 0.85 (t, 3H), 1.24 (m, 3H), 1.39-1.78 (m, 6H), 1.82 (dd, 1H), 1.98 (m, 2H), 2.20 (dd, 1H), 2.25 (m, 1H), 2.50 (m, 1H), 3.98 (s, 2H), 4.98 (dd, 2H), 7.18-7.40 (m, 10H), 7.45 (s, 1H), 7.98 (s, 1H), 8.23 (s, 1H), 8.42 (s, 1H).
55		30	^{1}H NMR ($CDCl_3$, 400MHz) δ: 0.80 (t, 3H), 0.92 (t, 3H), 1.21 (m, 2H), 1.30-1.70 (m, 12H), 1.82 (dd, 1H), 2.04 (m, 1H), 2.20 (m, 2H), 2.50 (m, 1H), 2.58 (t, 2H), 4.98 (dd, 2H), 6.83 (d, 1H), 7.30 (m, 5H), 7.90 (s, 1H), 8.08 (s, 1H), 8.15 (d, 1H).
56[2]		53	^{1}H NMR ($CDCl_3$, 300MHz) δ: 0.84 (t, 3H), 1.25 (m, 2H), 1.27-1.99 (m, 10H), 2.07-2.30 (m, 2H), 2.47 (m, 1H), 4.99 (s, 2H), 5.10 (dd, 2H), 6.59 (d, 1H), 7.15 (d, 1H), 7.34 (m, 11H), 8.10 (s, 1H).

1 = dichloromethane used as the column eluant

2 = N-methylmorpholine was used as the base

Preparation 57

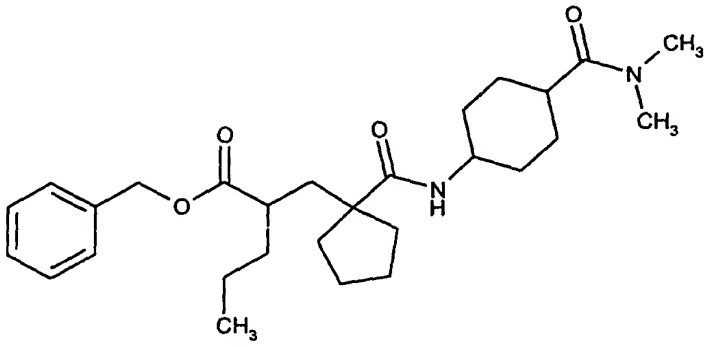
Benzyl 2-({1-[({1-benzyl-2-oxo-2-[(3-pyridinylsulfonyl)amino]ethyl}amino)-carbonyl]cyclopentyl}methyl)pentanoate



The amine hydrochloride from preparation 25 (828mg, 2.19mmol) and N-methylmorpholine (2.21g, 21.9mmol) was added to an ice-cold solution of the acid chloride from preparation 3 (737mg, 2.19mmol) in dichloromethane (50ml), and the reaction stirred at room temperature for 24 hours. The reaction mixture was evaporated under reduced pressure, the residue partitioned between ethyl acetate (50ml) and water (50ml), and the layers separated. The organic phase was washed with brine (25ml), dried ($MgSO_4$) and evaporated under reduced pressure. The crude product was purified by column chromatography on silica gel using an elution gradient of ethyl acetate:methanol (100:0 to 95:5) to give the title compound as a cream foam, 975mg, 73%; ^1H NMR ($CDCl_3$, 300MHz) δ: 0.72 (m, 3H), 0.94-2.20 (m, 17H), 2.84 (m, 1H), 3.00 (m, 1H), 4.18 (m, 1H), 5.00 (m, 2H), 6.95 (m, 2H), 7.02 (m, 3H), 7.38 (m, 6H), 8.06 (m, 1H), 8.60 (m, 1H), 8.87 (s, 1H).

Preparation 58

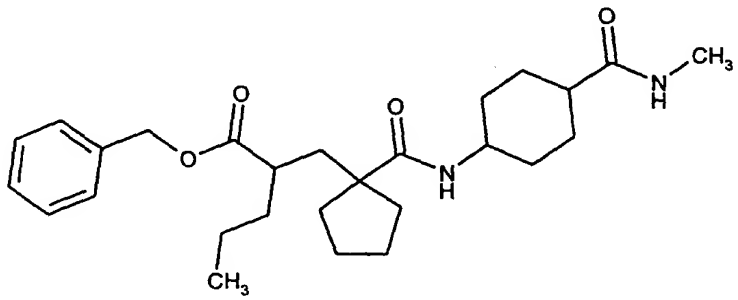
Cis-Benzyl 2-({1-[({4-[(dimethylamino)carbonyl]cyclohexyl}amino)carbonyl]-cyclopentyl}methyl)pentanoate



A mixture of cis-4-{[(1-{2-[(benzyloxy)carbonyl]pentyl}cyclopentyl)carbonyl]amino}-cyclohexanecarboxylic acid (EP 274234) (200mg, 0.45mmol), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (112mg, 0.58mmol), 1-hydroxybenzotriazole hydrate (70mg, 0.46mmol) and dimethylamine (0.56ml, 2M in tetrahydrofuran, 1.12mmol) in dichloromethane (5ml) was stirred at room temperature for 16 hours. The mixture was concentrated under reduced pressure and the residue partitioned between sodium bicarbonate solution and ethyl acetate, and the layers separated. The organic phase was dried ($MgSO_4$) and evaporated under reduced pressure to give a gum. The crude product was purified by column chromatography on silica gel using ethyl acetate as eluant to afford the title compound, 150mg; ^1H NMR ($CDCl_3$, 300MHz) δ: 0.82 (t, 3H), 1.22 (m, 3H), 1.32-1.88 (m, H), 2.00 (m, 4H), 2.40 (m, 1H), 2.60 (m, 1H), 2.97 (s, 3H), 3.04 (s, 3H), 4.04 (m, 1H), 5.12 (s, 2H), 5.80 (bd, 1H), 7.37 (m, 5H).

Preparation 59

Cis-Benzyl 2-({1-[({4-[(methylamino)carbonyl]cyclohexyl}amino)carbonyl]-cyclopentyl}methyl)pentanoate

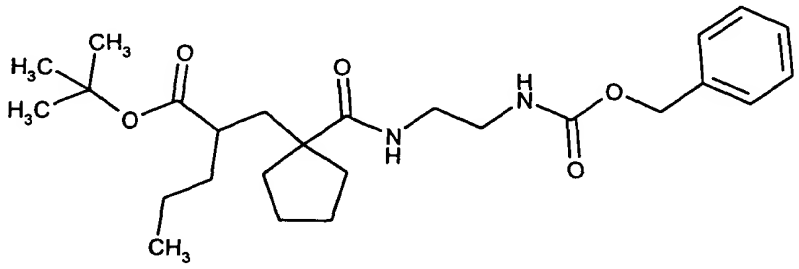


The title compound was prepared in 49% yield from cis-4-{[(1-{2-

[(benzyloxy)carbonyl]pentyl}cyclopentyl)carbonyl]amino}cyclohexanecarboxylic acid (EP 274234) and methylamine (2M in tetrahydrofuran), following the procedure described in preparation 58; ^{1}H NMR ($CDCl_3$, 300MHz) δ: 0.82 (t, 3H), 1.17-2.12 (m, 22H), 2.21 (m, 1H), 2.41 (m, 1H), 2.80 (d, 3H), 4.00 (m, 1H), 5.12 (s, 2H), 5.61 (m, 1H), 5.79 (d, 1H), 7.38 (m, 5H).

Preparation 60

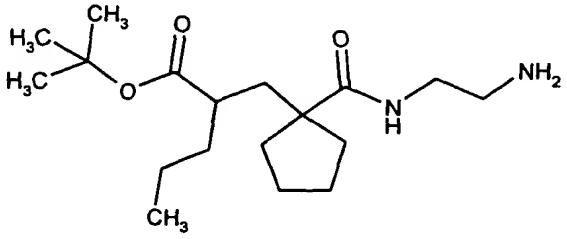
tert-Butyl 2-[(1-{[(2-{[(benzyloxy)carbonyl]amino}ethyl)amino]carbonyl}-cyclopentyl)methyl]pentanoate



The title compound was obtained as a yellow oil in 55% yield, from the acid from preparation 1 and N-benzyloxycarbonyl-1,2-diaminoethane, following a similar procedure to that described in preparation 44; ^{1}H NMR ($CDCl_3$, 400MHz) δ: 0.84 (t, 3H), 1.20-1.38 (m, 3H), 1.40-1.74 (m, 17H), 1.90 (m, 2H), 2.04 (m, 1H), 2.20 (m, 1H), 3.32 (m, 3H), 3.44 (m, 1H), 5.10 (s, 2H), 5.61 (m, 1H), 6.20 (m, 1H), 7.36 (m, 5H).

Preparation 61

tert-Butyl 2-[(1-{[(2-aminoethyl)amino]carbonyl}cyclopentyl)methyl]pentanoate

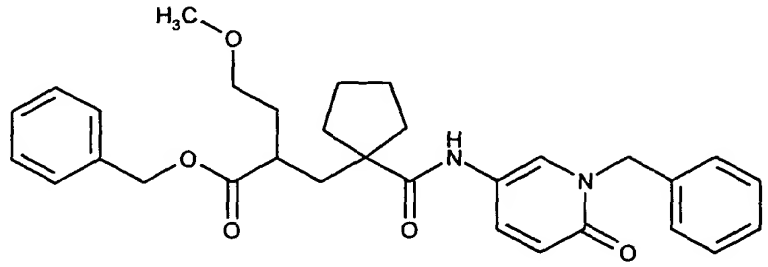


A mixture of the carbamate from preparation 60 (1.43g, 3.10mmol) and 10% palladium on charcoal (200mg) in ethanol (8ml) was hydrogenated at room temperature and 1 atm for 18 hours. The reaction mixture was filtered through Arbocel®, and the filtrate evaporated under reduced pressure to afford the title compound, 920mg, 92%; ^{1}H NMR ($CDCl_3$, 400MHz) δ: 0.84 (t, 3H), 1.20-1.38 (m, 3H), 1.40-1.54 (m, 12H), 1.61 (m, 5H), 1.92-2.12 (m, 3H), 2.20 (m, 1H), 2.98 (m, 2H), 3.38 (m, 1H), 3.42 (m, 1H), 3.97

(m, 2H), 6.65 (m, 1H); LRMS : m/z 326.8 (M^+).

Preparation 62

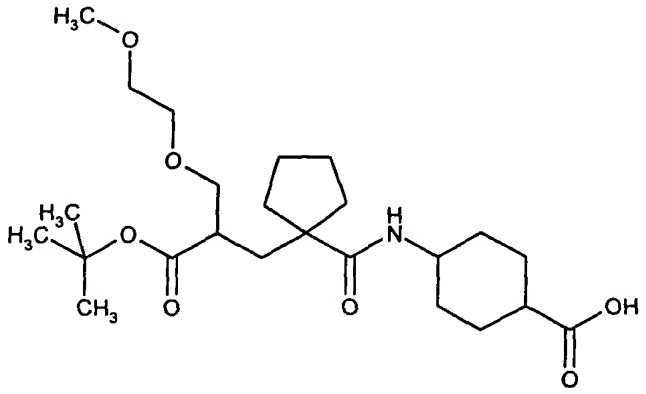
Benzyl 2-[(1-{[(1-benzyl-6-oxo-1,6-dihydro-3-pyridinyl)amino]carbonyl}cyclopentyl)-methyl]-4-methoxybutanoate



Oxalyl chloride (0.26ml, 3.0mmol) was added to an ice-cooled solution of 1-{2-[(benzyloxy)carbonyl]-4-methoxybutyl}cyclopentanecarboxylic acid (EP 274234) (1.0g, 3.0mmol) and N,N-dimethylformamide (2 drops) in dichloromethane (20ml), and the reaction stirred at room temperature for 2 hours. The solution was concentrated under reduced pressure and the residue azeotroped with dichloromethane (3x10ml). The product was dissolved in dichloromethane (20ml), then cooled in an ice-bath. The amine from preparation 28 (600mg, 3mmol) and N-methylmorpholine (0.6ml, 5.45mmol) were added and the reaction stirred at room temperature for 18 hours. The reaction mixture was concentrated under reduced pressure, and partitioned between water and ether. The organic layer was washed with hydrochloric acid (2N), sodium bicarbonate solution, then water, dried ($MgSO_4$) and evaporated under reduced pressure. The residual green solid was purified by medium pressure column chromatography on silica gel using ethyl acetate:hexane (90:10) as eluant to afford the title compound, 880mg, 57%; ^1H NMR ($CDCl_3$, 300MHz) δ: 1.37-2.28 (m, 12H), 2.46-2.64 (m, 1H), 3.20 (s, 3H), 3.31 (m, 2H), 4.97 (dd, 2H), 5.08 (dd, 2H), 6.57 (d, 1H), 7.12 (m, 1H), 7.18-7.48 (m, 10H), 8.08 (d, 1H).

Preparation 63

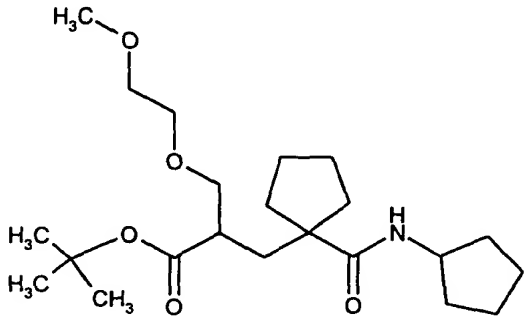
4-{[(1-{3-*tert*-Butoxy-2-[(2-methoxyethoxy)methyl]-3-oxopropyl}cyclopentyl)-carbonyl]amino}cyclohexanecarboxylic acid



A mixture of benzyl 4-{[(1-{3-*tert*-butoxy-2-[(2-methoxyethoxy)methyl]-3-oxopropyl}cyclopentyl)carbonyl]amino}cyclohexanecarboxylate (EP 274234), and 10% palladium on charcoal (250mg) in water (10ml) and ethanol (50ml) was hydrogenated at 50 psi and room temperature for 18 hours. The reaction mixture was filtered through Solkafloc®, the filtrate concentrated under reduced pressure and the residue azeotroped with toluene (3x) and then dichloromethane (3x), to give the title compound, 2.0g, 96%; ^{1}H NMR ($CDCl_3$, 300MHz) δ: 1.48 (s, 9H), 1.53-1.84 (m, 14H), 1.94-2.10 (m, 5H), 2.60 (m, 2H), 3.40 (s, 3H), 3.41-3.63 (m, 5H), 3.96 (m, 1H), 5.90 (bd, 1H).

Preparation 64

tert-Butyl 3-{1-[(cyclopentylamino)carbonyl]cyclopentyl}-2-[(2-methoxyethoxy)methyl]-propanoate

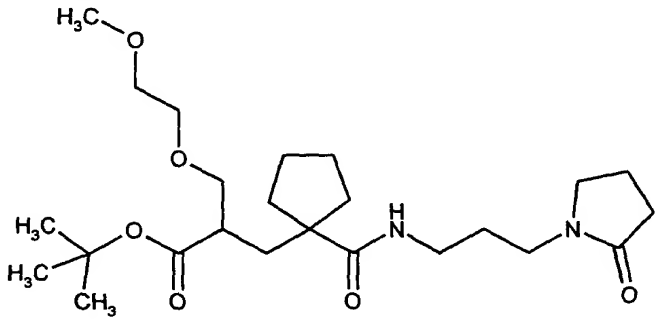


1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (197mg, 1.07mmol), 1-hydroxybenzotriazole hydrate (139mg, 1.07mmol), N-methylmorpholine (0.18ml, 1.64mmol) and cyclopentylamine (101µl, 1.07mmol) were added to a solution of 1-{3-*tert*-butoxy-2-[(2-methoxyethoxy)m thyl]-3-oxopropyl}-cyclopentanecarboxylic acid (EP

274234) (400mg, 1.07mmol) in dichloromethane (5ml), and the reaction stirred at room temperature for 22 hours. The reaction was quenched by the addition of water, extracted with dichloromethane (3x), and the combined organic extracts dried ($MgSO_4$) and evaporated under reduced pressure. The crude product was purified by column chromatography on silica gel using ethyl acetate:pentane (30:70) as eluant to afford the title compound as a clear oil, 320mg, 78%; ^{1}H NMR ($CDCl_3$, 400MHz) δ: 1.22-2.02 (m, 27H), 2.58 (m, 1H), 3.36 (s, 3H), 3.40 (m, 1H), 3.46 (m, 2H), 3.57 (m, 3H), 4.10-4.20 (m, 1H), 5.80 (bs, 1H).

Preparation 65

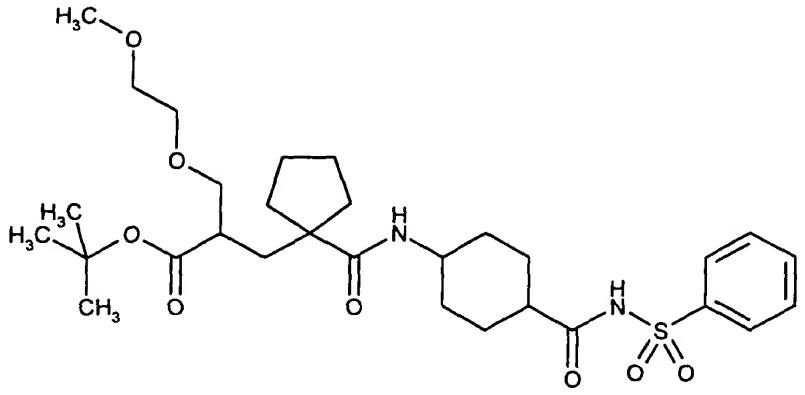
tert-Butyl 3-(2-methoxyethoxy)-2-{[1-({[3-(2-oxo-1-pyrrolidinyl)propyl]amino}carbonyl)cyclopentyl]methyl}propanoate



The title compound was obtained as a clear oil in 97% yield from 1-{3-*tert*-butoxy-2-[(2-methoxyethoxy)methyl]-3-oxopropyl}-cyclopentanecarboxylic acid (EP 274234) and 1-(3-aminopropyl)-2-pyrrolidinone, following a similar procedure to that described in preparation 64, except dichloromethane:methanol (95:5) was used as the column eluant, ^{1}H NMR ($CDCl_3$, 400MHz) δ: 1.41 (s, 9H), 1.50 (m, 2H), 1.60-1.70 (m, 7H), 1.78 (m, 1H), 1.90 (m, 1H), 2.20 (m, 4H), 2.40 (m, 2H), 2.58 (m, 1H), 3.14 (m, 1H), 3.20 (m, 1H), 3.38 (m, 6H), 3.42-3.60 (m, 6H), 7.00 (m, 1H).

Preparation 66

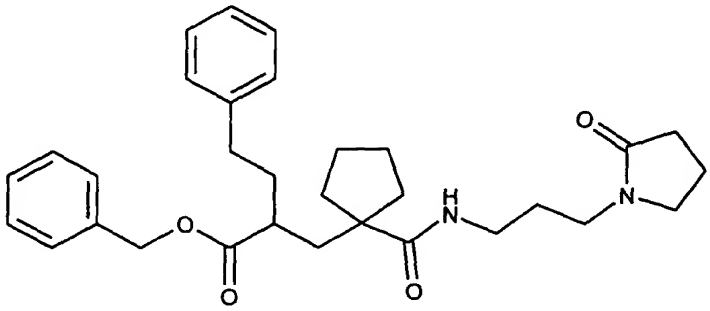
Cis-*tert*-Butyl 3-(2-methoxyethoxy)-2-[(1-{[(4-{[(phenylsulfonyl)amino]carbonyl}-cyclohexyl)amino]carbonyl}cyclopentyl)methyl]propanoate



N,N'-Dicyclohexylcarbodiimide (199mg, 0.97mmol), 4-dimethylaminopyridine (118mg, 0.97mmol) and benzenesulphonamide (152mg, 0.97mmol) were added to an ice-cooled solution of the acid from preparation 63 (400mg, 0.878mmol) in dichloromethane (12ml) and N,N-dimethylformamide (0.5ml), and the reaction stirred at room temperature for 20 hours. The mixture was concentrated under reduced pressure and the residue suspended in cold ethyl acetate. The resulting insoluble material was filtered off, the filtrate washed with hydrochloric acid (1N), and water, then dried ($MgSO_4$) and evaporated under reduced pressure. The crude product was purified by column chromatography on silica gel using an elution gradient of dichloromethane:methanol (95:5 to 90:10) to afford the title compound as a white foam, 480mg, 92%; ^1H NMR ($CDCl_3$, 400MHz) δ: 1.44 (s, 9H), 1.63 (m, 13H), 1.80 (m, 2H), 1.88 (m, 1H), 1.98 (m, 2H), 2.36 (m, 1H), 2.57 (m, 1H), 3.38 (s, 3H), 3.40 (m, 1H), 3.51 (t, 2H), 3.58 (m, 3H), 3.95 (m, 1H), 5.92 (d, 1H), 7.56 (m, 2H), 7.62 (m, 1H), 8.05 (d, 2H), 8.75 (bs, 1H); LRMS : m/z 618 (MNa^+).

Preparation 67

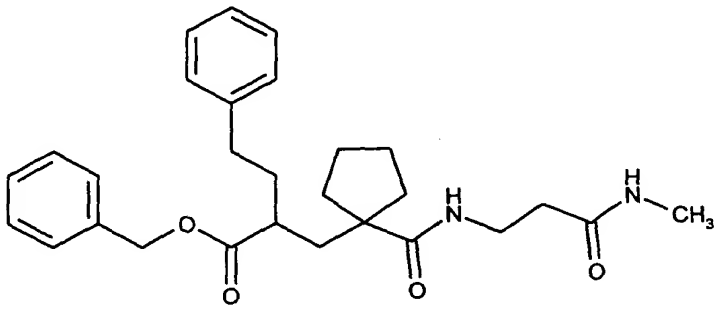
Benzyl 2-{[1-({[3-(2-Oxo-1-pyrrolidinyl)propyl]amino}carbonylcyclopentyl]-methyl}-4-phenylbutanoate



1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (1.06g, 5.53mmol), 1-hydroxybenzotriazole hydrate (0.60g, 4.44mmol) and 4-methylmorpholine (0.56g, 5.54mmol) were added sequentially to a cooled solution of 1-{2-[(benzyloxy)carbonyl]-4-phenylbutyl}cyclopentane-carboxylic acid (EP 274234) (1.5g, 3.94mmol) in dry dichloromethane (15ml) at room temperature, followed by N-(3-aminopropyl)-2-pyrrolidinone (0.56g, 3.94mmol), and the reaction stirred at room temperature for 18 hours. The mixture was washed with water, 2N hydrochloric acid, saturated aqueous sodium bicarbonate solution, and then dried ($MgSO_4$) and evaporated under reduced pressure. The residual yellow oil was purified by column chromatography on silica gel using ethyl acetate:pentane (50:50) as the eluant to provide the title compound as a clear gum, 800mg, 40%; ^1H NMR ($CDCl_3$, 300MHz) d : 1.37-2.20 (m, 16H), 2.34-2.58 (m, 5H), 2.92-3.46 (m, 6H), 5.07 (d, 1H), 5.18 (d, 1H), 6.98-7.47 (m, 10H).

Preparation 68

Benzyl 2-{[1-({[3-(methylamino)-3-oxopropyl]amino}carbonyl)cyclopentyl]methyl}-4-phenylbutanoate

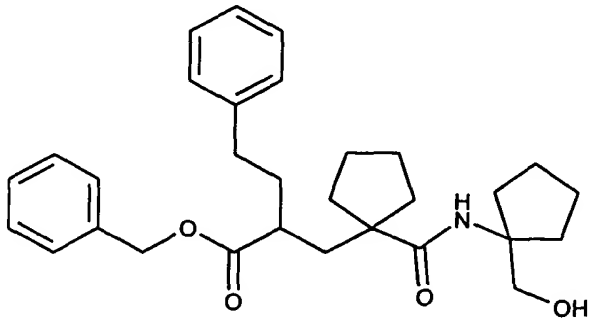


1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (122mg, 0.64mmol), 1-hydroxybenzotriazole hydrate (86mg, 0.64mmol) and 4-methylmorpholine (173µl,

1.59mmol) were added sequentially to a cooled solution of 1-{2-[(benzyloxy)carbonyl]-4-phenylbutyl}cyclopentane-carboxylic acid (EP 274234) (202mg, 0.53mmol) in N,N-dimethylformamide (5ml) at room temperature, followed by the amine hydrochloride from preparation 23 (146mg, 1.06mmol), and the reaction stirred at 90°C for 18 hours. The cooled solution was concentrated under reduced pressure and the residue partitioned between water (20ml) and ethyl acetate (100ml). The layers were separated, the organic phase washed with water (3x30ml), brine (25ml) dried ($MgSO_4$), and evaporated under reduced pressure to give a clear oil. The crude product was purified by column chromatography on silica gel using dichloromethane:methanol (98:2) as eluant to afford the title compound as a colourless oil, 162mg, 67%; ^1H NMR ($CDCl_3$, 400MHz) δ: 1.38-1.53 (m, 2H), 1.53-1.96 (m, 8H), 2.02 (m, 2H), 2.27 (t, 2H), 2.46 (m, 3H), 2.76 (d, 3H), 3.44 (m, 2H), 5.13 (s, 2H), 5.79 (bs, 1H), 6.38 (m, 1H), 7.06 (d, 2H), 7.18 (m, 1H), 7.22 (m, 2H), 7.38 (m, 5H); LRMS : m/z 465.5 (MH^+).

Preparation 69

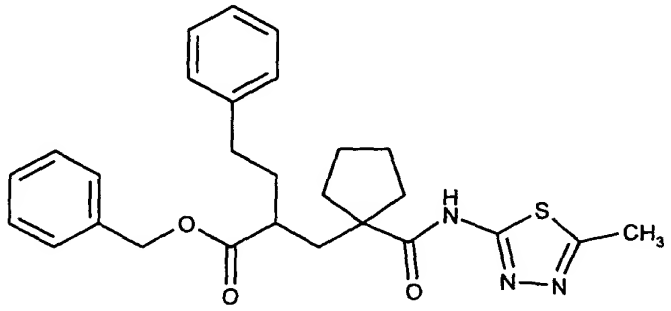
Benzyl 2-{[1-({[1-(hydroxymethyl)cyclopentyl]amino}carbonyl)cyclopentyl]methyl}-4-phenylbutanoate



The title compound was obtained as a crystalline solid (48%) from 1-{2-[(benzyloxy)carbonyl]-4-phenylbutyl}cyclopentane-carboxylic acid (EP 274234) and 1-amino-1-cyclopentanemethanol, following a similar procedure to that described in preparation 68, except the reaction mixture was stirred at room temperature for 18 hours, and the crude product purified by column chromatography on silica gel using ethyl acetate:pentane as eluant; ^1H NMR ($CDCl_3$, 400MHz) δ: 1.38 (m, 2H), 1.50-1.95 (m, 16H), 2.01 (m, 2H), 2.45 (m, 3H), 3.49 (dd, 1H), 3.60 (dd, 1H), 4.58 (m, 1H), 5.10 (s, 2H), 5.67 (s, 1H), 7.01 (d, 2H), 7.14 (m, 1H), 7.20 (m, 2H), 7.36 (m, 5H); LRMS : m/z 478.3 (MH^+).

Preparation 70

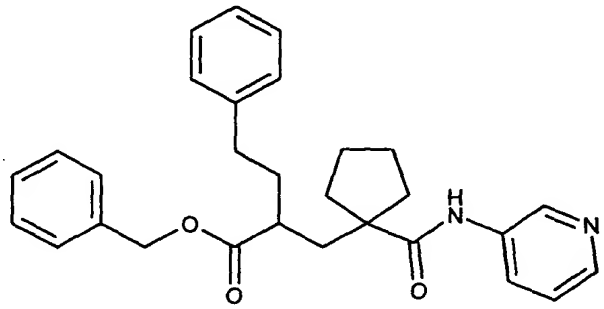
Benzyl 2-[(1-{[(5-methyl-1,3,4-thiadiazol-2-yl)amino]carbonyl}cyclopentyl)methyl]-4-phenylbutanoate



The title compound was obtained as a clear oil in 74% yield from 1-{2-[(benzyloxy)carbonyl]-4-phenylbutyl}cyclopentane-carboxylic acid (EP 274234) and 2-amino-5-methyl-1,3,4-thiadiazole, following a similar procedure to that described in preparation 68; ^{1}H NMR ($CDCl_3$, 400MHz) δ: 1.58-1.76 (m, 7H), 1.83-1.98 (m, 3H), 2.03 (m, 1H), 2.20 (m, 1H), 2.35 (m, 1H), 2.44 (m, 3H), 2.65 (s, 3H), 5.02 (dd, 2H), 7.00 (d, 2H), 7.15 (m, 1H), 7.19 (m, 2H), 7.35 (m, 5H); LRMS : m/z 478.7 (MH^+).

Preparation 71

Benzyl 4-phenyl-2-({1-[(3-pyridinylamino)carbonyl]cyclopentyl}methyl)butanoate



Oxalyl chloride (2.29ml, 26.3mmol) was added to a solution of 1-{2-[(benzyloxy)carbonyl]-4-phenylbutyl}cyclopentane-carboxylic acid (EP 274234) (5.0g, 13.14mmol) and N,N-dimethylformamide (2 drops) in dichloromethane (25ml), and the solution stirred for 2.5 hours. The mixture was evaporated under reduced pressure, the residue azeotroped with dichloromethane to give a yellow oil. This was then dissolved in dichloromethane (50ml) and a solution of this acid chloride (10ml, 2.45mmol) was added to an ice-cooled solution of triethylamine (248mg, 2.45mmol) and 3-aminopyridine (253mg, 2.70mmol) in dry dichloromethane (10ml), and the reaction stirred at room temperature for 18 hours. The solution was washed with water

(3x), dried ($MgSO_4$) and evaporated under reduced pressure. The crude product was purified by column chromatography on silica gel using ethyl acetate:hexane (40:60) as eluant, and repeated using an elution gradient of ether:hexane (90:10 to 100:0). The product was crystallised from ethyl acetate:hexane to afford the title compound, 740mg, 66%; ^1H NMR ($CDCl_3$, 300MHz) δ: 1.38-2.07 (m, 10H), 2.10-2.37 (m, 2H), 2.42-2.63 (m, 3H), 5.02 (s, 2H), 6.94-7.44 (m, 10H), 7.50 (s, 1H), 8.03 (d, 1H), 8.36 (d, 1H), 8.52 (s, 1H).

NEP Assay:

The Preparation and Assay of Soluble Neutral Endopeptidase (NEP) from Canine, Rat, Rabbit and Human Kidney Cortex.

Soluble NEP is obtained from the kidney cortex and activity is assayed by measuring the rate of cleavage of the NEP substrate Abz-D-Arg-Arg-Leu-EDDnp to generate its fluorescent product, Abz-D-Arg-Arg.

Experimental Procedure:-

1 Materials

All water is double de ionised.

1.1 Tissues:

Human Kidney	IIAM (Pennsylvania. U.S.A.)
Rat Kidney	In house tissue supply
Rabbit Kidney	In house tissue supply
Canine Kidney	In house tissue supply

1.2 Homogenisation medium:

100mM Mannitol and 20mM Tris @ pH 7.1

2.42g Tris (Fisher T/P630/60) is diluted in 1 litre of water and the pH adjusted to 7.1 using 6M HCl at room temperature. To this 18.22g Mannitol (Sigma M-9546) is added.

1.3 Tris buffer (NEP buffer):

50ml of 50mM Tris pH 7.4 (Sigma T2663) is diluted in 950ml of water.

1.4 Substrate (Abz-D-Arg-Arg-Leu-EDDnp):

Made to order from SNPE, and is stored as a powder at –20°C. A 2mM stock is made by gently re-suspending the substrate in Tris buffer, this should not be vortexed or sonicated. 600µl aliquots of the 2mM stock are stored at –20 for up to one month. (Medeiros, M.A.S., Franca,

M.S.F. et al., (1997), Brazilian Journal of Medical and Biological Research, 30, 1157-1162).

1.5 Total product:

Samples corresponding to 100% substrate to product conversion are included on the plate to enable the % substrate turnover to be determined. The total product is generated by incubating 1ml of 2mM substrate with 20µl of enzyme stock for 24 hours at 37°C.

1.6 Stock solution:

A 300µM stock of Phosphoramidon (Sigma R7385) is made up in NEP buffer and stored in 50µl aliquots at -20.

1.7 Dimethyl sulphoxide (DMSO).

1.8 Magnesium Chloride -$MgCl_2.6H_2O$ (Fisher M0600/53).

1.9 Black 96 well flat bottom assay plates (Costar 3915).

1.10 Topseal A (Packard 6005185).

1.11 Centrifuge tubes

2 Specific Equipment

2.1 Sorvall RC-5B centrifuge (SS34 GSA rotor, pre-cooled to 4°C).

2.2 Braun miniprimer mixer.

2.3 Beckman CS-6R centrifuge.

2.4 Fluostar galaxy.

2.5 Wesbart 1589 shaking incubator.

3 Methods

3.1 Tissue Preparation

3.2 Dog, rat, rabbit, and human NEP is obtained from the kidney cortex using a method adapted from Booth, A.G. & Kenny, A.J. (1974) *Biochem. J.* 142, 575-581.

3.3 Frozen kidneys are allowed to thaw at room temperature and the cortex is dissected away from the medulla.

3.4 The cortex is finely chopped and homogenised in approximately 10 volumes of homogenisation buffer (1.2) using a Braun miniprimer (2.2).

3.5 Magnesium chloride (1.8) (20.3mg/gm tissue) is added to the homogenate and stirred in an ice-water bath for 15 minutes.

3.6 The homogenate is centrifuged at 1,500g (3,820rpm) for 12 minutes in a

Beckman centrifuge (2.3) before removing the supernatant to a fresh centrifuge tube and discarding the pellet.

3.7 The supernatant is centrifuged at 15,000g (12,100rpm) for 12 minutes in a Sovall centrifuge (2.1) and the supernatant is discarded.

3.8 The pale pink layer on the top of the remaining pellet is removed and re-suspended in homogenisation buffer containing magnesium chloride (9mg MgCl in 5ml buffer per 1g tissue).

3.9 The suspension is centrifuged at 2,200g (4,630rpm) for 12 minutes in a Beckman centrifuge (2.3) before discarding the pellet.

3.10 The supernatant is centrifuged at 15,000g (12,100rpm) for 12 minutes using the Sorvall centrifuge (2.1) and the supernatant is discarded.

3.11 The final pellet is resuspended in homogenisation buffer containing magnesium chloride (0.9mg MgCl in 0.5ml buffer per 1g tissue). A homogenous suspension is obtained using a Braun miniprimer (2.2). This is then frozen down in 100µl aliquots to be assayed for NEP activity.

4 Determination of NEP Activity

The activity of the previously aliquoted NEP is measured by its ability to cleave the NEP specific peptide substrate.

4.1 A 4% DMSO/NEP buffer solution is made (4mls DMSO in 96mls NEP buffer).

4.2 Substrate, total product, enzyme, and Phosphoramidon stocks are left on ice to thaw.

4.3 50µl of 4% DMSO/NEP buffer solution is added to each well.

4.4 The 2mM substrate stock is diluted 1:40 to make a 50µM solution. 100µl of 50µM substrate is added to each well (final concentration 25µM).

4.5 50µl of a range of enzyme dilutions is added to initiate the reaction (usually 1:100, 1:200, 1:400, 1:800, 1:1600, and 1:3200 are used). 50µl of NEP buffer is added to blank wells.

4.6 The 2mM total product is diluted 1:80 to make a 25µM solution. 200µl of 25µM product is added to the first four wells of a new plate.

4.7 Plates are incubated at 37oC in a shaking incubator for 60 minutes.

4.8 The 300µM Phosphoramidon stock is diluted 1:100 to 300nM. The reaction is stopped by the addition of 100µl 300nM Phosphoramidon and incubated at 37°C in a shaking incubator for 20 minutes before being read on the Fluostar (ex320/em420).

5 NEP Inhibition Assays

5.1 Substrate, total product, enzyme and Phoshoramidon stocks are left on ice to thaw.

5.2 Compound stocks are made up in 100% DMSO and diluted 1:25 in NEP buffer to give a 4% DMSO solution. All further dilutions are carried out in a 4% DMSO solution (4mls DMSO in 96mls NEP buffer).

5.3 50µl of compound in duplicate is added to the 96 well plate and 50µl of 4% DMSO/NEP buffer is added to control and blank wells (see appendix for plate layout). Alternatively see appendix for robotic dilutions.

5.4 The 2mM substrate stock is diluted 1:40 in NEP buffer to make a 50µM solution (275µl 2mM substrate to 10.73ml buffer is enough for 1 plate).

5.5 The enzyme stock diluted in NEP buffer (determined from activity checks).

5.6 The 2mM total product stock is diluted 1:80 in NEP buffer to make a 25µM solution. 200µl is added to the first four wells of a separate plate.

5.7 The 300µM Phosphoramidon stock is diluted 1:1000 to make a 300nM stock (11µl Phosphoramidon to 10.99ml NEP buffer.

5.8 To each well in the 96 well plate the following is added:

Table: Reagents to be added to 96 well plate.

	Compound/ DMSO	Tris Buffer	Substrate	NEP enzyme	Total product
Samples	2µl compound	50µl	100µl	50µl	None
Controls	2µl DMSO	50µl	100µl	50µl	None
Blanks	2µl DMSO	100µl	100µl	None	None
Totals	2µl DMSO	None	None	None	200µl

5.9 The reaction is initiated by the addition of the NEP enzyme before incubating at 37°C for 1 hour in a shaking incubator.

5.10 The reaction is stopped with 100µl 300nM Phosphoramidon and incubated at 37°C for 20 minutes in a shaking incubator before being read on the Fluostar (ex320/em420).

6 Calculations

The activity of the NEP enzyme is determined in the presence and absence of compound and expressed as a percentage.

% Control activity (turnover of enzyme) =

$$\frac{\text{Mean FU of controls} - \text{Mean FU of blanks}}{\text{Mean FU of totals} - \text{Mean FU of blanks}} \times 100$$

% Activity with inhibitor =

$$\frac{\text{Mean FU of compound} - \text{Mean FU of blanks}}{\text{Mean FU of totals} - \text{Mean FU of blanks}} \times 100$$

Activity expressed as % of control =

$$\frac{\text{\% Activity with inhibitor}}{\text{\% Control activity}} \times 100$$

A sigmoidal dose-response curve is fitted to the % activities (% of control) vs compound concentration and IC50 values calculated using LabStats fit-curve in Excel.

The specific examples herein all had an IC50 against NEP of less than 5000nM.

In addition (in a preferred embodiment) many of the examples tested also had a selectivity for NEP over ACE of at least 300 fold.

ACE Assay

The Preparation and Assay of Soluble Angiotensin Converting Enzyme (Ace), from Porcine and Human Kidney Cortex.

Soluble ACE activity is obtained from the kidney cortex and assayed by measuring the rate of cleavage of the ACE substrate Abz-Gly-p-nitro-Phe-Pro-OH to generate its fluorescent product, Abz-Gly.

1 Materials

All water is double de ionised.

1.1 Human Kidney: IIAM (Pennsylvania. U.S.A.) or UK Human

Tissue Bank (UK HTB)

1.2 Porcine kidney ACE Sigma (A2580)

1.3 Homogenisation buffer-1

100mM Mannitol and 20mM Tris @ pH 7.1

2.42g Tris (Fisher T/P630/60) is diluted in 1 litre of water and the pH adjusted to 7.1 using 6M HCl at room temperature. To this 18.22g Mannitol (Sigma M-9546) is added.

1.4 Homogenisation buffer-2

100mM Mannitol, 20mM Tris @ pH7.1 and 10mM $MgCl_2.6H_2O$ (Fisher M0600/53)

To 500ml of the homogenisation buffer 1 (1.4) 1.017g of $MgCl_2$ is added.

1.5 Tris buffer (ACE buffer).

50mM Tris and 300mM NaCl @ pH 7.4

50ml of 50mM Tris pH 7.4 (Sigma T2663) and 17.52g NaCl (Fisher S/3160/60) are made up to 1000ml in water.

1.6 Substrate (Abz-D-Gly-p-nitro-Phe-Pro-OH) (Bachem M-1100)

ACE substrate is stored as a powder at –20°C. A 2mM stock is made by gently re-suspending the substrate in ACE buffer, this must not be vortexed or sonicated. 400µl aliquots of the 2mM stock are stored at –20°C for up to one month.

1.7 Total product

Samples corresponding to 100% substrate to product conversion are included on the plate to enable the % substrate turnover to be determined (see calculations). The total product is generated by incubating 1ml of 2mM substrate with 20µl of enzyme stock for 24 hours at 37°C.

1.8 Stop solution.

0.5M EDTA (Promega CAS[6081/92/6]) is diluted 1:250 in ACE buffer to make a 2mM solution.

1.9 Dimethyl sulphoxide (DMSO).

1.10 Magnesium Chloride -$MgCl_2.6H_2O$ (Fisher M0600/53).

1.11 Black 96 well flat bottom assay plates (Costar 3915 or Packard).

1.12 Topseal A (Packard 6005185).

1.13 Centrifuge tubes

2 <u>Specific Equipment</u>

2.1 Sorvall RC-5B centrifuge (SS34 GSA rotor, pre-cooled to 4°C).

2.2 Braun miniprimer mixer.

2.3 Beckman CS-6R centrifuge.

2.4 BMG Fluostar Galaxy.

2.5 Wesbart 1589 shaking incubator.

3 Methods

3.1 Tissue Preparation

3.2 Human ACE is obtained from the kidney cortex using a method adapted from Booth, A.G. & Kenny, A.J. (1974) *Biochem. J.* 142, 575-581.

3.3 Frozen kidneys are allowed to thaw at room temperature and the cortex is dissected away from the medulla.

3.4 The cortex is finely chopped and homogenised in approximately 10 volumes of homogenisation buffer-1 (1.4) using a Braun miniprimer (2.2).

3.5 Magnesium chloride (1.11) (20.3mg/gm tissue) is added to the homogenate and stirred in an ice-water bath for 15 minutes.

3.6 The homogenate is centrifuged at 1,500g (3,820rpm) for 12 minutes in a Beckman centrifuge (2.3) before removing the supernatant to a fresh centrifuge tube and discarding the pellet.

3.7 The supernatant is centrifuged at 15,000g (12,100rpm) for 12 minutes in a Sovall centrifuge (2.1) and the supernatant is discarded.

3.8 The pale pink layer on the top of the remaining pellet is removed and re-suspended in homogenisation buffer-2 (1.5) (5ml buffer per 1g tissue).

3.9 The suspension is centrifuged at 2,200g (4,630rpm) for 12 minutes in a Beckman centrifuge before discarding the pellet.

3.10 The supernatant is centrifuged at 15,000g (12,100rpm) for 12 minutes using the Sorvall centrifuge and the supernatant is discarded.

3.11 The final pellet is resuspended in homogenisation buffer-2 (0.5ml buffer per 1g tissue). A homogenous suspension is obtained using a Braun miniprimer. This is then frozen down in 100µl aliquots to be assayed for NEP activity.

4 Determination Of ACE Activity

The activity of the previously aliquoted ACE is measured by its ability to cleave the ACE specific peptide substrate.

Porcine ACE (1.2) is defrosted and resuspended in ACE buffer (1.6) at

0.004U/µl, this is frozen down in 50µl aliquots.

4.1 A 4% DMSO/ACE buffer solution is made (4mls DMSO in 96mls ACE buffer).

4.2 Substrate (1.7), total product (1.8) and enzyme (1.1, 1.2, 1.3), are left on ice to thaw.

4.3 50µl of 4% DMSO/ACE buffer solution is added to each well.

4.4 The 2mM substrate stock is diluted 1:100 to make a 20µM solution. 100µl of 20µM substrate is added to each well (final concentration in the assay 10µM).

4.5 50µl of a range of enzyme dilutions is added to initiate the reaction (usually 1:100, 1:200, 1:400, 1:800, 1:1600, and 1:3200 are used). 50µl of ACE buffer is added to blank wells.

4.6 The 2mM total product is diluted 1:200 to make 10µM solution. 200µl 10µM product is added to the first four wells of a new plate.

4.7 Plates are incubated at 37°C in a shaking incubator for 60 minutes.

4.8 The enzyme reaction is stopped by the addition of 100µl 2mM EDTA in ACE buffer and incubated at 37°C in a shaking incubator for 20 minutes before being read on the BMG Fluostar Galaxy (ex320/em420).

5 <u>ACE Inhibition Assays</u>

5.1 Substrate, total product, and enzyme stocks are left on ice to thaw.

5.2 Compound stocks are made up in 100% DMSO and diluted 1:25 in ACE buffer to give a 4% DMSO solution. All further dilutions are carried out in a 4% DMSO/ACE buffer solution (4mls DMSO in 96mls ACE buffer).

5.3 50µl of compound, in duplicate, is added to the 96 well plate and 50µl of 4% DMSO/ACE buffer is added to control and blank wells (see appendix-1 for plate layout).

5.4 Steps 5.2 and 5.3 can be carried out either by hand or using the Packard multiprobe robots (see appendix-2 for details)

5.5 The 2mM substrate stock is diluted 1:100 in ACE buffer to make a 20µM solution (10µM final concentration in the assay) (110µl of 2mM substrate added to 10.89ml buffer is enough for 1 plate).

5.6 The enzyme stock is diluted in ACE buffer, as determined from activity checks (4.0).

5.7 The 2mM total product stock is diluted 1:200 in ACE buffer to make a 10µM solution. 200µl is added to the first four wells of a separate plate.

5.8 The 0.5mM EDTA stock is diluted 1:250 to make a 2mM stock (44µl EDTA to

10.96ml ACE buffer).

5.9 To each well of the 96 well plate the following reagents are added:

Table 1: Reagents added to 96 well plate.

	Compound/ DMSO	Tris Buffer	Substrate	ACE enzyme	Total product
Samples	2µl compound	50µl	100µl	50µl	None
Controls	2µl DMSO	50µl	100µl	50µl	None
Blanks	2µl DMSO	100µl	100µl	None	None
Totals	2µl DMSO	None	None	None	200µl

5.10 50µl of the highest concentration of each compound used in the assay is added in duplicate to the same 96 well plate as the totals (5.7). 150µl of ACE buffer is added to determine any compound fluorescence.

5.11 The reaction is initiated by the addition of the ACE enzyme before incubating at 37°C for 1 hour in a shaking incubator.

5.12 The reaction is stopped by the addition of 100µl 2mM EDTA and incubated at 37°C for 20 minutes in a shaking incubator, before being read on the BMG Fluostar Galaxy (ex320/em420).

6 Calculations

The activity of the ACE enzyme is determined in the presence and absence of compound and expressed as a percentage. (FU = Fluorescence units)

(i) % Control activity (turnover of enzyme) =

$$\frac{\text{Mean FU of controls} - \text{Mean FU of blanks}}{\text{Mean FU of totals} - \text{Mean FU of blanks}} \times 100$$

(ii) % Activity with inhibitor =

$$\frac{\text{Mean FU of compound} - \text{Mean FU of blanks}}{\text{Mean FU of totals} - \text{Mean FU of blanks}} \times 100$$

(iii) Activity expressed as % of control =

$$\frac{\text{\% Activity with inhibitor}}{\text{\% Control activity}} \times 100$$

or

$$\frac{\text{Mean FU of compound} - \text{Mean FU of blanks}}{\text{Mean FU of controls} - \text{Mean FU of blanks}} \times 100$$

(iv) % Inhibition = 100 - % control

(v) For fluorescent compounds the mean FU of blanks containing compound (5.10) is deducted from the mean FU of compound values used to calculate the % Activity.

A sigmoidal dose-response curve is fitted to the % activities (% of control) vs compound concentration and IC_{50} values calculated using LabStats fit-curve in Excel.

Animal Model of arousal response

A particularly preferred compound of the invention (selected from the list of 10 compounds given herebefore) was administered according to the following protocol to show an increase in genital blood flow in the rabbit (it has previously been shown by Ottensen that an increase in vaginal blood flow increase vaginal lubrication-. Ottesen, B., Pedersen, B., Nielsen, J. *et al.* (1987). Vasoactive intestinal polypeptide (VIP) provokes vaginal lubrication in normal women. *Peptides*, 8, 797-800.

"The genital organs consist of an internal and external group. The internal organs are situated within the pelvis and consist of ovaries, the uterine tubes, uterus and the vagina. The external organsare superficial to the urogenital diaphragm and below the pelvic arch. They comprise the mons pubis, the labia majora and minora pudendi, the clitoris, the vestibule, the bulb of the vestibule, and the greater vestibular glands" (Gray's Anatomy, C.D. Clemente, 13th American Edition).

Methods

Anaesthetic Protocol

Female New Zealand rabbits (~2.5kg) were pre-medicated with a combination of Medetomidine (Domitor®) 0.5ml/kg *i.m.*, and Ketamine (Vetalar®) 0.25ml/kg *i.m.* whilst maintaining oxygen intake via a face mask. The rabbits were tracheotomised using a Portex™ uncuffed endotracheal tube 3 ID., connected to ventilator and maintained a ventilation rate of 30-40 breaths per minute, with an approximate tidal volume of 18-20 ml, and a maximum airway pressure of 10 cm H_2O. Anaesthesia was then switched to Isoflurane and ventilation continued with O_2 at 2l/min. The right marginal ear vein was cannulated using a 23G or 24G catheter, and Lactated Ringer solution perfused at 0.5ml/min. The rabbit was maintained at 3% Isoflurane during invasive surgery, dropping to 2% for maintenance anaesthesia.

Cannulation of Vessels

The left groin area of the rabbit was shaved and a vertical incision was made approximately 5cm in length along the thigh. The femoral vein and artery were exposed, isolated and then cannulated with a PVC catheter (17G) for the infusion of drugs and compounds. Cannulation was repeated for the femoral artery, inserting the catheter to a depth of 10cm to ensure that the catheter reached the abdominal aorta. This arterial catheter was linked to a Gould system to record blood pressure. Samples for blood gas analysis were also be taken via the arterial catheter. Systolic and diastolic pressures were measured, and the mean arterial pressure calculated using the formula (diastolic x2 + systolic) ÷3. Heart rate was measured via the pulse oxymeter and *Po-ne-mah* data acquisition software system (Ponemah Physiology Platform, Gould Instrument Systems Inc).

Stimulation of the Pelvic Nerve

A ventral midline incision was made into the abdominal cavity. The incision was about 5cm in length just above the pubis. The fat and muscle was *bluntly* dissected away to reveal the hypogastric nerve which runs down the body cavity. It was essential to keep close to the side curve of the pubis wall in order to avoid damaging the femoral vein and artery which lie above the pubis. The sciatic and pelvic nerves lie deeper and were located after further dissection on the dorsal side of the rabbit. Once the sciatic nerve is identified, the pelvic nerve was easily located. The term *pelvic nerve* is loosely applied; anatomy books on the subject fail to identify the nerves in sufficient detail.

However, stimulation of the nerve causes an increase in vaginal and clitoral blood flow, and innervation of the pelvic region. The pelvic nerve was freed away from surrounding tissue and a *Harvard* bipolar stimulating electrode was placed around the nerve. The nerve was slightly lifted to give some tension, then the electrode was secured in position. Approximately 1ml of light paraffin oil was placed around the nerve and electrode. This acts as a protective lubricant to the nerve and prevents blood contamination of the electrode. The electrode was connected to a *Grass* S88 Stimulator. The pelvic nerve was stimulated using the following parameters:- 5V, pulse width 0.5ms, duration of stimulus 10 seconds and a frequency range of 2 to 16Hz. Reproducible responses were obtained when the nerve was stimulated every 15-20 minutes.

A frequency response curve was determined at the start of each experiment in order to determine the optimum frequency to use as a sub-maximal response, normally 4Hz. The compound(s) to be tested were infused, via the femoral vein, using a *Harvard* 22 infusion pump allowing a continuous 15 minute stimulation cycle.

Positioning of the Laser Doppler Probes

A ventral midline incision was made, at the caudal end of the pubis, to expose the pubic area. Remove any connective tissue, and expose the tunica of the clitoris, ensuring that the wall is free from small blood vessels. The external vaginal wall was also exposed by removing any connective tissue. One laser Doppler flow probe was inserted 3cm into the vagina, so that half the probe shaft is still visible. A second probe was positioned so that it lies just above the external clitoral wall. The position of these probes was then adjusted until a signal was obtained. A second probe was placed just above the surface of a blood vessel on the external vaginal wall. Both probes were clamped in position.

Vaginal and clitoral blood flow was recorded either as numbers directly from the Flowmeter using *Po-ne-mah* data acquisition software (Ponemah Physiology Platform, Gould Instrument Systems Inc), or indirectly from Gould chart recorder trace. Calibration is set at the beginning of the experiment (0-125ml/min/100g tissue).

Infusion of Inhibitors

A particularly preferred NEP (Neutral Endopeptidase EC3.4.24.11) inhibitor chosen from the list of 10 given hereinbefore was made up in saline or 5% glucose solution (200µl 50% glucose in 1.8ml water for injection). The inhibitor and vehicle controls were infused using a Harvard 22 pump, infusing at 500µl/min via a 3-way tap into the femoral vein. After the infusion, the catheter was flushed with heparinised saline (Hepsaline) so that no NEP inhibitor was left in the catheter.

Results and Discussion

Animal model of sexual arousal

The major cause of FSAD is decreased genital blood flow and this manifests itself as reduced vaginal, labial and clitoral engorgement. Treatment of women with FSAD is achievable by restoration of the *normal* sexual arousal response. This can be achieved by enhancing genital blood flow.

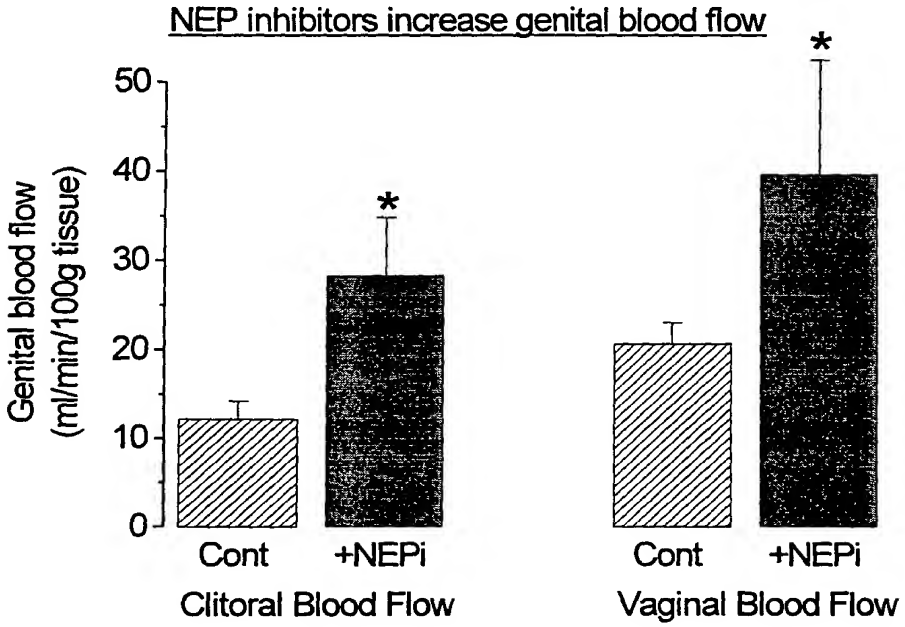
We have developed a robust reproducible model of the physiology of sexual arousal. Using this anaesthetised rabbit model, we are capable of measuring small changes in genital blood flow using Laser Doppler technology. Stimulation of the pelvic nerve is used to simulate the neuronal effects of sexual arousal.
FSAD is associated with and may result from reduced genital blood flow.

The selective NEP inhibitor tested, at clinically relevant doses, significantly enhanced pelvic nerve stimulated increases in genital blood flow (See Figure 1). The NEP inhibitor enhanced the peak increase in vaginal blood flow by up to 92% (n=3) and clitoral blood flow by 131% (n=3) compared to time matched control increases.

We have developed an animal model that mimics the physiological arousal response observed during female sexual arousal and directly reflects the clinical data obtained in human volunteers. The model uses Laser Doppler technologies to record small changes in vaginal and clitoral blood flow induced by pelvic nerve stimulation or vasoactive neurotransmitters. During sexual arousal, there is an increase in genital blood flow resulting from increased innervation from the pelvic nerve. The pelvic nerve-stimulated increase in vaginal and clitoral blood flow, observed in the animal model, represents the endogenous vascular effects observed during female sexual arousal. Therefore this model can be used to firstly, identify the mechanisms involved in the

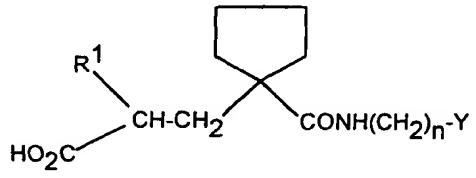
regulation of vaginal and clitoral blood flow and secondly, use the model to validate novel approaches for the enhancement of genital blood flow.

Figure 1:- The selective inhibitor of NEP EC 3.4.24.11 enhanced pelvic nerve stimulated (PNS) increases in genital blood flow in the anaesthetised rabbit model of sexual arousal. Repetitive PNS at 15 minute intervals induced reproducible increases in genital blood flow (Hatched Bars). Administration of a NEP inhibitor (Grey bar) enhanced the peak increase in clitoral and vaginal blood flow induced by submaximal stimulation frequencies (eg 4Hz) compared to increases observed during time matched control stimulations or vehicle controls (Hatched bar). The following simultaneous enhancements were observed following a 1.0mg/kg iv bolus – a 131% increase in clitoral and a 92% increase in vaginal blood flow (n=3). Data expressed as mean ± sem; all changes were monitored using laser Doppler technologies.



Claims

1 The use of a compound of formula I (or a pharmaceutically acceptable salt, solvate or prodrug thereof) in the preparation of a medicament for the treatment of sexual dysfunction:



(I)

wherein

R^1 is C_{1-6}alkyl which may be substituted by one or more substituents, which may be the same or different, selected from the list: halo, hydroxy, C_{1-6} alkoxy, C_{2-6} hydroxyalkoxy, C_{1-6}alkoxy(C_{1-6}alkoxy), C_{3-7}cycloalkyl, C_{3-7}cycloalkenyl, aryl, aryloxy, (C_{1-4}alkoxy)aryloxy, heterocyclyl, heterocyclyloxy, $-NR^2R^3$, $-NR^4COR^5$, $-NR^4SO_2R^5$, $-CONR^2R^3$, $-S(O)_pR^6$, $-COR^7$ and $-CO_2(C_{1-4}$alkyl); or R^1 is C_{3-7}cycloalkyl, aryl or heterocyclyl, each of which may be substituted by one or more substituents from said list, which substituents may be the same or different, which list further includes C_{1-6}alkyl; or R^1 is C_{1-6} alkoxy, $-NR^2R^3$ or $-NR^4SO_2R^5$;

wherein

R^2 and R^3 are each independently H, C_{1-4}alkyl, C_{3-7}cycloalkyl (optionally substituted by hydroxy or C_{1-4}alkoxy), aryl, (C_{1-4}alkyl)aryl, C_{1-6}alkoxyaryl or heterocyclyl; or R^2 and R^3 together with the nitrogen to which they are attached form a pyrrolidinyl, piperidino, morpholino, piperazinyl or *N*-(C_{1-4} alkyl)piperazinyl group;

R^4 is H or C_{1-4}alkyl;

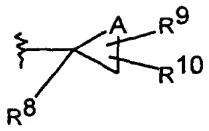
R^5 is C_{1-4}alkyl, CF_3, aryl, (C_{1-4} alkyl)aryl, (C_{1-4}alkoxy)aryl, heterocyclyl, C_{1-4}alkoxy or $-NR^2R^3$ wherein R^2 and R^3 are as previously defined;

R^6 is C_{1-4}alkyl, aryl, heterocyclyl or NR^2R^3 wherein R^2 and R^3 are as previously defined; and

R^7 is C_{1-4}alkyl, C_{3-7}cycloalkyl, aryl or heterocyclyl; n is 0, 1 or 2; p is 0, 1, 2 or 3;

the $-(CH_2)_n-$ linkage is optionally substituted by C_{1-4}alkyl, C_{1-4}alkyl substituted with one or more fluoro groups or phenyl, C_{1-4}alkoxy, hydroxy, hydroxy(C_{1-3}alkyl), C_{3-7}cycloalkyl, aryl or heterocyclyl;

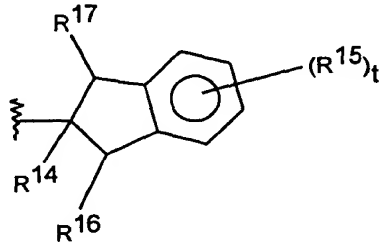
Y is the group



wherein A is $-(CH_2)_q-$ where q is 1, 2, 3 or 4 to complete a 3 to 7 membered carbocyclic ring which may be saturated or unsaturated; R^8 is H, C_{1-6}alkyl, $-CH_2OH$, phenyl, phenyl(C_{1-4}alkyl) or $CONR^{11}R^{12}$; R^9 and R^{10} are each independently H, $-CH_2OH$, $-C(O)NR^{11}R^{12}$, C_{1-6}alkyl, phenyl optionally substituted by C_{1-4}alkyl, or phenyl(C_{1-4}alkyl) wherein the phenyl group is optionally substituted by C_{1-4}alkyl, or R^9 and R^{10} together form a dioxolane; $R^{11}$and R^{12} which may be the same or different are H, C_{1-4}alkyl, R^{13} or $S(O)_rR^{13}$, where r is 0, 1 or 2 and R^{13} is phenyl optionally substituted by C_{1-4}alkyl or phenylC_{1-4}alkyl wherein the phenyl is optionally substituted by C_{1-4}alkyl; or

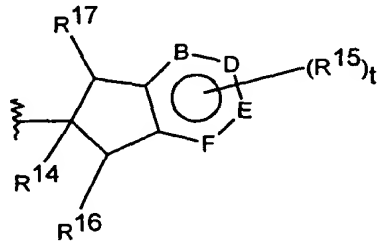
Y is the group, $-C(O)\ NR^{11}\ R^{12}$ wherein R^{11} and R^{12} are as previously defined except that R^{11} and R^{12} are not both H; or

Y is the group,



wherein R^{14} is H, CH_2OH, or $C(O)NR^{11}R^{12}$ wherein R^{11} and R^{12} are as previously defined; when present R^{15}, which may be the same or different to any other R^{15}, is OH, C_{1-4}alkyl, C_{1-4}alkoxy, halo or CF_3; t is 0, 1, 2, 3 or 4; and R^{16} and R^{17} are independently H or C_{1-4} alkyl; or

Y is the group



wherein one or two of B, D, E or F is a nitrogen, the others being carbon; and

R^{14} to R^{17} and t are as previously defined; or

Y is an optionally substituted 5-7 membered heterocyclic ring, which may be saturated, unsaturated or aromatic and contains a nitrogen, oxygen or sulphur and optionally one, two or three further nitrogen atoms in the ring and which may be optionally benzofused and optionally substituted by:

C_{1-6} alkoxy; hydroxy; oxo; amino; mono or di-(C_{1-4}alkyl)amino; C_{1-4}alkanoylamino; or

C_{1-6}alkyl which may be substituted by one or more groups, which may be the same or different, selected from the list: C_{1-6}alkoxy, C_{1-6}haloalkoxy, C_{1-6}alkylthio, halogen, C_{3-7}cycloalkyl, heterocyclyl or phenyl; or

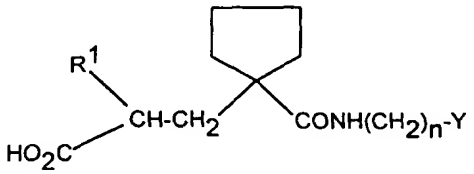
C_{3-7}cycloalkyl, aryl or heterocyclyl, each of which may be substituted by one or more groups, which may be the same or different, selected from the list: C_{1-6}alkyl, C_{1-6}alkoxy, C_{1-6}haloalkoxy, C_{1-6}alkylthio, halogen, C_{3-7}cycloalkyl, heterocyclyl or phenyl;

wherein when there is an oxo substitution on the heterocyclic ring, the ring only contains one or two nitrogen atoms and the oxo substitution is adjacent a nitrogen atom in the ring; or

Y is $-NR^{18}S(O)_uR^{19}$, wherein R^{18} is H or C_{1-4}alkyl; R^{19} is aryl, arylC_{1-4}alkyl or heterocyclyl; and

u is 0, 1, 2 or 3.

2 A compound of formula (I), or a pharmaceutically acceptable salt, solvate or prodrug thereof



(I)

wherein

R^1 is C_{1-6}alkyl which may be substituted by one or more substituents, which may be the same or different, selected from the list: halo, hydroxy, C_{1-6} alkoxy, C_{2-6} hydroxyalkoxy, C_{1-6}alkoxy(C_{1-6}alkoxy), C_{3-7}cycloalkyl, C_{3-7}cycloalkenyl, aryl, aryloxy, (C_{1-4}alkoxy)aryloxy, heterocyclyl, heterocyclyloxy, $-NR^2R^3$, $-NR^4COR^5$, $-NR^4SO_2R^5$, $-CONR^2R^3$, -

$S(O)_pR^6$, $-COR^7$ and $-CO_2(C_{1-4}alkyl)$; or R^1 is C_{3-7}cycloalkyl, aryl or heterocyclyl, each of which may be substituted by one or more substituents from said list, which substituents may be the same or different, which list further includes C_{1-6}alkyl; or R^1 is C_{1-6} alkoxy, $-NR^2R^3$ or $-NR^4SO_2R^5$;

wherein

R^2 and R^3 are each independently H, C_{1-4}alkyl, C_{3-7}cycloalkyl (optionally substituted by hydroxy or C_{1-4}alkoxy), aryl, (C_{1-4}alkyl)aryl, C_{1-6}alkoxyaryl or heterocyclyl; or R^2 and R^3 together with the nitrogen to which they are attached form a pyrrolidinyl, piperidino, morpholino, piperazinyl or *N*-(C_{1-4} alkyl)piperazinyl group;

R^4 is H or C_{1-4}alkyl;

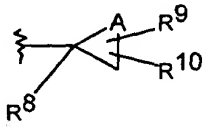
R^5 is C_{1-4}alkyl, CF_3, aryl, (C_{1-4} alkyl)aryl, (C_{1-4}alkoxy)aryl, heterocyclyl, C_{1-4}alkoxy or $-NR^2R^3$ wherein R^2 and R^3 are as previously defined;

R^6 is C_{1-4}alkyl, aryl, heterocyclyl or NR^2R^3 wherein R^2 and R^3 are as previously defined; and

R^7 is C_{1-4}alkyl, C_{3-7}cycloalkyl, aryl or heterocyclyl; n is 0, 1 or 2; p is 0, 1, 2 or 3;

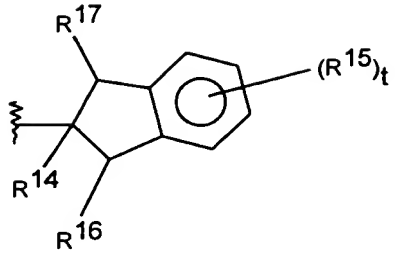
the $-(CH_2)_n-$ linkage is optionally substituted by C_{1-4}alkyl, C_{1-4}alkyl substituted with one or more fluoro groups or phenyl, C_{1-4}alkoxy, hydroxy, hydroxy(C_{1-3}alkyl), C_{3-7}cycloalkyl, aryl or heterocyclyl;

Y is the group



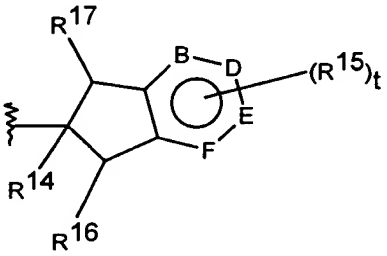
wherein A is $-(CH_2)_q-$ where q is 1, 2, 3 or 4 to complete a 3 to 7 membered carbocyclic ring which may be saturated or unsaturated; R^8 is H, C_{1-6}alkyl, $-CH_2OH$, phenyl, phenyl(C_{1-4}alkyl) or $CONR^{11}R^{12}$; R^9 and R^{10} are each independently H, $-CH_2OH$, $-C(O)NR^{11}R^{12}$, C_{1-6}alkyl, phenyl optionally substituted by C_{1-4}alkyl, or phenyl(C_{1-4}alkyl) wherein the phenyl group is optionally substituted by C_{1-4}alkyl, or R^9 and R^{10} together form a dioxolane; $R^{11}$and R^{12} which may be the same or different are H, C_{1-4}alkyl, R^{13} or $S(O)_rR^{13}$, where r is 0, 1 or 2 and R^{13} is phenyl optionally substituted by C_{1-4}alkyl or phenylC_{1-4}alkyl wherein the phenyl is optionally substituted by C_{1-4}alkyl; or

Y is the group,



wherein R^{14} is $C(O)NR^{11}R^{12}$ wherein R^{11} and R^{12} are as previously defined; when present R^{15}, which may be the same or different to any other R^{15}, is OH, C_{1-4}alkyl, C_{1-4}alkoxy, halo or CF_3; t is 0, 1, 2, 3 or 4; and R^{16} and R^{17} are independently H or C_{1-4} alkyl; or

Y is the group



wherein one or two of B, D, E or F is a nitrogen, the others being carbon; and R^{14} to R^{17} and t are as previously defined; or

Y is an optionally substituted 5-7 membered heterocyclic ring, which may be saturated, unsaturated or aromatic and contains a nitrogen, oxygen or sulphur and optionally one, two or three further nitrogen atoms in the ring and which may be optionally benzofused and optionally substituted by:

C_{1-6} alkoxy; hydroxy; oxo; amino; mono or di-(C_{1-4}alkyl)amino; C_{1-4}alkanoylamino; or

C_{1-6}alkyl which may be substituted by one or more groups, which may be the same or different, selected from the list: C_{1-6}alkoxy, C_{1-6}haloalkoxy, C_{1-6}alkylthio, halogen, C_{3-7}cycloalkyl, heterocyclyl or phenyl; or

C_{3-7}cycloalkyl, aryl or heterocyclyl, each of which may be substituted by one or more groups, which may be the same or different, selected from the list: C_{1-6}alkyl, C_{1-6}alkoxy, C_{1-6}haloalkoxy, C_{1-6}alkylthio, halogen, C_{3-7}cycloalkyl, heterocyclyl or phenyl;

wherein when there is an oxo substitution on the heterocyclic ring, the

ring only contains one or two nitrogen atoms and the oxo substitution is adjacent a nitrogen atom in the ring; or

Y is $-NR^{18}S(O)_uR^{19}$, wherein R^{18} is H or C_{1-4}alkyl; R^{19} is aryl, arylC_{1-4}alkyl or heterocyclyl; and

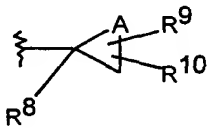
u is 0, 1, 2 or 3.

3 A compound as defined in claims 1 or 2 wherein R^1 is C_{1-6}alkyl, C_{1-6}alkoxy or C_{1-6}alkyl substituted with aryl.

4 A compound as defined in claim 3 wherein R^1 is C_{1-6}alkyl, C_{1-6}alkoxy or C_{1-6}alkoxy(C_{1-3})alkyl.

5 A compound as defined in claim 4 wherein R^1 is C_{1-4}alkyl.

6 A compound as defined in any one of the preceding claims wherein when Y is the group



and the carbocyclic ring is fully saturated, then one of R^9 or R^{10} is $-CH_2OH$, $-C(O)NR^{11}R^{12}$, C_{1-6}alkyl, phenyl optionally substituted by C_{1-4}alkyl or phenyl(C_{1-4}alkyl) wherein the phenyl group is optionally substituted by C_{1-4}alkyl.

7 A compound as defined in claim 6 wherein the carbocyclic ring is 5, 6 or 7 membered wherein one of R^9 or R^{10}, $-C(O)NR^{11}R^{12}$, with the other being C_{1-6}alkyl, phenyl optionally substituted by C_{1-4}alkyl or phenyl(C_{1-4}alkyl) wherein the phenyl group is optionally substituted by C_{1-4}alkyl.

8 A compound as defined in claim 7 wherein R^9 and R^{10} are attached to adjacent carbon atoms in the ring.

9 A compound as defined in any one of the preceding claims wherein R^8 is CH_2OH.

10 A compound as defined in any of claims 1 to 5 wherein when Y is the group - $NR^{18}S(O)_uR^{19}$, then R^{18} is H.

11 A compound as defined in any of claims 1 to 5 or 10 wherein R^{19} is benzyl or phenyl.

12 A compound as defined in any of claims 1 to 5 , 10 or 11 wherein u is 2.

13 A compound as defined in claim 1 wherein Y is an optionally substituted 5-7 membered heterocyclic ring, which may be saturated, unsaturated or aromatic and contains a nitrogen, oxygen or sulphur and optionally one, two or three further nitrogen atoms in the ring and which may be optionally benzofused and optionally substituted by one or more of C_{1-6} alkyl, phenyl, phenylC_{1-4}alkyl, C_{1-6} alkoxy, hydroxy, oxo, amino, mono or di-(C_{1-4}alkyl)amino or C_{1-4} alkanoylamino; with the proviso that when there is an oxo substitution, the ring only contains one or two nitrogen atoms and the oxo substitution is adjacent a nitrogen atom in the ring.

14 A compound as defined in claim 13 wherein the heterocyclic ring is a heteroaromatic ring.

15 A compound as defined in claim 14 wherein the heteroaromatic ring is selected from pyridyl, pyrazinyl, pyrimidinyl, pyridazinyl, pyrazolyl, triazolyl, tetrazolyl, oxadiazole, thiazole, thiaiazole, oxazolyl, isoxazolyl, indolyl, isoindolinyl, quinolyl, pyridone, quinoxalinyl, and quinazolinyl each being optionally substituted as defined in claim 1.

16 A compound as defined in claim 15 wherein the heteroaromatic ring is selected from oxadiazole, pyridone and thiadiazole.

17 A compound as defined in claim 16 wherein the heteroaromatic ring is selected from 1,2,5 oxadiazole, 1,3,4 oxadiazole, 2-pyridone and 1,3,4 thiadiazole.

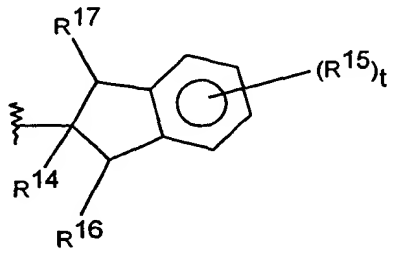
18 A compound as defined in any of claims 13 to 17 wherein the heterocyclic ring is substituted by one or more C_{1-6}alkyl, phenyl or phenylC_{1-4}alkyl.

19 A compound as defined in claim 18 wherein the substitution is C_{1-4}alkyl or benzyl.

20 A compound as defined in claims 16 to 19 wherein when Y is a pyridone, said pyridone is *N*-substituted (preferably with benzyl or C_{1-4}alkyl).

21 A compound as defined in claim 13 wherein Y is a lactam linked at the nitrogen.

22 A compound as defined in claim 1 and claims dependent thereon wherein Y is



wherein R^{14} is H, CH_2OH or $C(O)NR^{11}R^{12}$, R^{15} is one or more of H, OH, C_{1-4}alkyl, C_{1-4}alkoxy, halo or CF_3; and R^{16} and R^{17} are independently H or C_{1-4}alkyl.

23 A compound as defined in claim 22 wherein R^{14} is CH_2OH or $C(O)NR^{11}R^{12}$.

24 A compound as defined in claim 23 wherein R^{14} is $C(O)NR^{11}R^{12}$.

25 A compound as defined in claims 2 and claims 22 to 24 wherein R^{16} and R^{17} are H.

26 A compound as defined in claims 2 and claims 21 to 24 wherein t is 0.

27 A compound according to any one of the preceding claims selected from the group consisting of:

2-[(1-{[(1-benzyl-6-oxo-1,6-dihydro-3-pyridinyl)amino]carbonyl}-cyclopentyl)methyl]-4-methoxybutanoic acid (Example 35),

2-{[1-({[3-(2-oxo-1-pyrrolidinyl)propyl]amino}carbonylcyclopentyl]-methyl}-4-phenylbutanoic acid (Example 40),

(+)-2-{[1-({[2-(hydroxymethyl)-2,3-dihydro-1*H*-inden-2-yl]amino}carbonyl)cyclopentyl]-methyl}-4-phenylbutanoic acid (Example 44),
2-[(1-{[(5-methyl-1,3,4-thiadiazol-2-yl)amino]carbonyl}cyclopentyl)-methyl]-4-phenylbutanoic acid (Example 43),
cis-3-(2-methoxyethoxy)-2-[(1-{[(4-{[(phenylsulfonyl)amino]carbonyl}-cyclohexyl)amino]carbonyl}cyclopentyl)methyl]propanoic acid (Example 38),
(+)-2-{[1-({[2-(hydroxymethyl)-2,3-dihydro-1*H*-inden-2-yl]amino}carbonyl)-cyclopentyl]methyl}pentanoic acid (Example 31),
(+)-2-[(1-{[(5-ethyl-1,3,4-thiadiazol-2-yl)amino]carbonyl}cyclopentyl)-methyl]pentanoic acid (Example 30),
2-({1-[(3-benzylanilino)carbonyl]cyclopentyl}methyl)pentanoic acid (Example 21),
2-[(1-{[(1-benzyl-6-oxo-1,6-dihydro-3-pyridinyl)amino]carbonyl}cyclopentyl)-methyl]-pentanoic acid (Example 22), and
2-{[1-({[(1R,3S,4R)-4-(aminocarbonyl)-3-butylcyclohexyl]amino}carbonyl)-cyclopentyl]methyl}pentanoic acid (Example 9).

28 A compound as defined in any one of the preceding claims wherein the chiral carbon attached to R^1 is preferably the R-enantiomer.

29 Use of a compound as defined in claim 2 and claims dependent thereon in the preparation of a medicament for the treatment or prophylaxis of a condition for which a beneficial therapeutic response can be obtained by the inhibition of neutral endopeptidase.

30 Use according to claim 29 for the treatment or prophylaxis of sexual dysfunction.

31 Use of a compound according to claims 1 or 30 wherein the sexual dysfunction treated is female sexual dysfunction.

32 Use according to claim 31 wherein the female sexual dysfunction(s) treated includes at least female sexual arousal dysfunction.

33 Use according to any one of claims 30 to 32 wherein the medicament is administered systemically.

34 Use according to claim 33 wherein the medicament is administered orally.

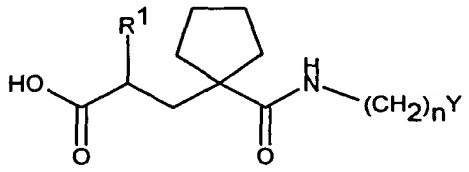
35 A compound as defined in claim 2 and claims dependent thereon for use in medicine.

36 A pharmaceutical formulation including a compound as defined in claim 2 and claims dependent thereon together with a pharmaceutically acceptable excipient.

37 A method for the treatment or prophylaxis of sexual dysfunction including administering to the patient a therapeutically effective amount of a compound as defined in any one of claims 1 to 28.

38 A sexual dysfunction pharmaceutical formulation including a therapeutically effective amount of a compound as defined in any one of claims 1 to 28 together with a pharmaceutically acceptable excipient.

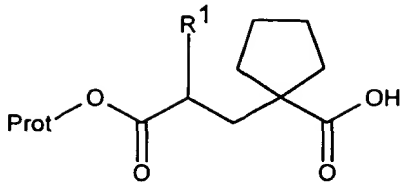
39 A process for preparing of a compound of formula I or salts thereof



(I)

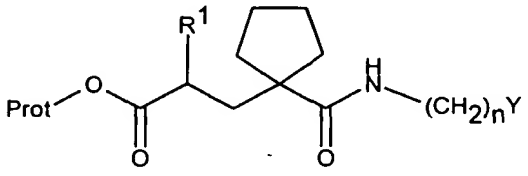
wherein R^1, n and Y are as defined in claim 2, comprising the steps of:

a) reacting a compound of formula II



(II)

wherein Prot is a suitable protecting group, with a compound of formula $Y(CH_2)_nNH_2$ (III), to give a compound of formula IV,



(IV)

then

b) reacting the compound of formula IV under suitable deprotecting conditions to give the compound of formula I; then

c) optionally forming a salt.